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STUDIES ON PARASITES OF THE ORIENTAL FRUIT MOTH

I. TRICHOGRAMMA

J. C. SCHREAD and PHILIP GARMAN

Connecticut
Agricultural Experiment Station
New Haven

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STUDIES ON PARASITES OF THE ORIENTAL FRUIT MOTH[1]

I. TRICHOGRAMMA

J. C. Schread and Philip Garman

Parasite breeding and liberation is a popular subject among fruit growers because of the apparent ease of control compared to the use of insecticides, but the difficulties in the way of successful application of the method are many and varied. The present studies have been made with a view to solving some of them, particularly those connected with production and distribution. In addition, we have had opportunities to study the effect of various insecticides upon the parasites under consideration and to watch the effect of field liberations in different Connecticut orchards. We also present a number of life history studies as well as some biological data, chiefly of scientific interest.

It has been necessary to study carefully the effects of laboratory breeding upon available hosts of the parasites and this has led in the case of Trichogramma to adoption of a breeding method that has been particularly successful and economical of materials. We have distributed to growers since 1930 more than 35 million Trichogramma egg parasites.

Discussion of field control work has been shortened in this paper (1) because satisfactorily controlled experiments are difficult to obtain, due to the interference of natural parasitism as well as climatological factors, and (2) because it has been necessary to devote so much time to production and shipment of the parasites that adequate field studies have been impossible in any one season. A few notes are given, however, which show the general trend of affairs in regard to Trichogramma parasitism at this time, but we believe much more remains to be done along the line of biological control in both field and laboratory before this is a reliable means of combating the Oriental fruit moth under all orchard conditions. We offer this bulletin merely as a contribution toward that end.

[1] *Grapholitha molesta* Busck.

HOSTS FOR BREEDING

Eleven or more hosts have been used by investigators to breed various strains or species of Trichogramma. The flour, meal, and fig moths, *Ephestia* spp., and the meal snout moth, *Pyralis farinalis,* have all been studied or used experimentally. The main trouble with these hosts seems to lie in the destructive habits of the larvae (Flanders[1]), which destroy unhatched eggs after emergence, or in the difficulties of mass production. Eggs of the following have also been used but how successfully is not mentioned in the available literature: *Cimex lectularis, Corcyra cephalonica,* the bee moth *Galleria mellonella,* and several noctuid moths. The bagworm, *Thyridopteryx ephemeraeformis* Haw., common on evergreens in the southern portions of the United States, has been the subject of considerable investigation (Peterson[2]) and shows promise for production work. The most generally used host, however, is the Angoumois grain moth, *Sitotroga cereallela* Oliv., though it is now being recognized that hosts laying larger eggs are more desirable from several angles. Nevertheless, it is more easily adapted to laboratory production than any of the others and enormous quantities of eggs may be obtained with proper manipulation.

THE GRAIN MOTH

LIFE HISTORY

The life history of the grain moth has been studied extensively by many workers so that it will be necessary to mention here only the main facts and points that affect mass production.

The incubation period of the egg varies from four and one-half to six days at 80° to 85° F. and 70 per cent relative humidity. The eggs are white when freshly laid, but turn to a pinkish color 24 hours afterwards. There are five larval instars in wheat, and the total larval period lasts 17 days at 80° F. and 70 per cent relative humidity. The adults live variable lengths of time, depending on how they are handled. At 80° F., 70 per cent relative humidity, they lay 90 per cent of their eggs within two days. Each female will lay eight to 14 eggs when bred in wheat, but are known to lay an average of 40 when reared in corn. In our cages the minimum life cycle, egg to adult at 87°, 75 per cent humidity, was 24 days; at 76°, 41 days.

PRODUCTION

FOOD, TEMPERATURE, AND INOCULATION OF UNITS

Wheat has been used in grain moth production at the Connecticut Station because of the ease of handling it in our units, the speed of

[1] Jour. Econ. Ent., 33: 603-606. 1930.
[2] U. S. Dept. Agr. Tech., Bul. 215. 1930.

development, and the greater number of eggs and moths apparently produced. The breeding units shown in Figure 139 are provided with grain and placed in a small room (10 by 10 by 7 ft.) which is then heated to a high temperature. This destroys potential enemies of the grain moth or other grain feeding insects which might compete with it for food. Afterwards, the temperature is lowered to

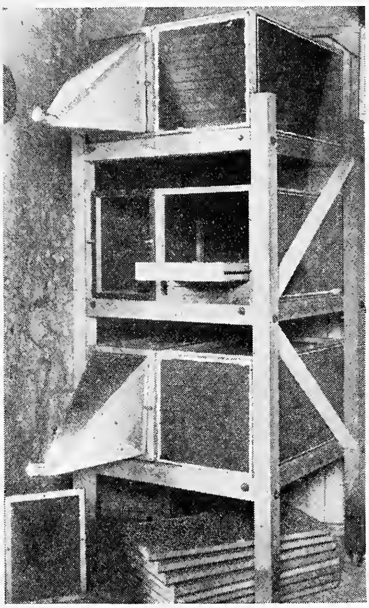

FIGURE 139. Grain moth unit for rearing grain moths. Empty compartment at left is used as a reservoir into which moths are blown before blowing through funnel into oviposition can. See also Fig. 140.

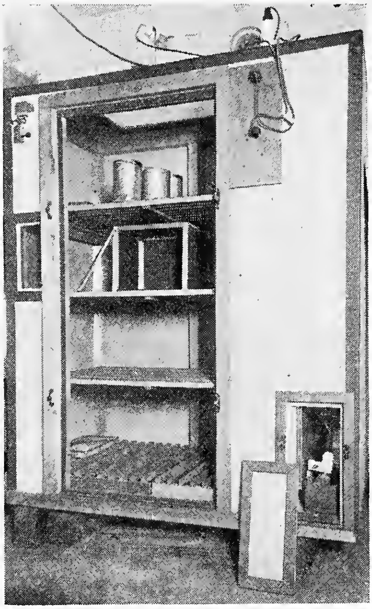

FIGURE 140. Incubator for oviposition cans of *Angoumois* grain moth. Dark box for Trichogramma on middle shelf.

80° F. and the humidity raised to 70 per cent, where it is held for about a week before the breeding unit is inoculated with grain moth eggs. This is accomplished by placing cards of eggs in the trays until the eggs hatch, after which the egg cards are removed. High moisture is essential in the room during the winter and especially during the hatching period of the eggs used to start the infestation. The grain is softened by moisture so the young larvae penetrate more easily.

The most satisfactory temperature for rearing grain moths appears to be 80° to 85° F. Seventy to 75 per cent humidity is desirable.

After inoculation, it is necessary to allow the cages to remain undisturbed for two or three generations (48 to 72 days), depending on the degree of initial inoculation, before removing the moths. If the introduction is heavy enough (50,000 eggs per unit), the emergence will be sufficient to allow removal within the specified time, and the moths may be blown out at stated intervals (three times a week) without depleting the supply. It is necessary to allow a day or so between removal of moths so that the grain will be restocked with eggs from those in the cages.

EXTRACTION OF MOTHS, OVIPOSITION, AND SEPARATION OF EGGS

The extraction of moths is accomplished by means of a portable blower giving a strong blast of air. By this means, the moths are forced into the empty compartment at the left of the unit (Figure 141) and from there into a tin oviposition can attached to the end of the funnel. Thorough removal of moths from the trays is desirable because they are the ones laying the most eggs.

Our oviposition cans (Figure 145, F.) are provided with short stilts and 30-mesh strainer cloth, which proved more satisfactory than other grades of wire cloth because it prevents escape of the moths and allows scales or other foreign matter to be removed quickly. The wire of this screen is about 36 guage and the stilts are about one-fourth inch long. When filled with the required number of moths, the oviposition cans are placed on tin trays—six cans to a tray—in an 80° F. incubator where the humidity is 60 per cent. A small electric fan supplies a circulation of air to clean away the scales, dust, and hairs that fall from the moths through the mesh and collect on the trays underneath the cans.

At first cornstarch was thought to induce oviposition and a fine layer was sifted over the surface of the trays before the moth cans were placed on them in the incubator, but cornstarch has been found to be of no advantage in this respect and its use consequently discontinued. The important factor favoring egg deposition by grain moths is a crowded condition in the oviposition cans. The more moths per can, within a limit governed by the size of the can, the more eggs will be laid. If there are but a few moths in an oviposition can, which allows for their unhampered movement, each female will deposit fewer eggs.

Thousands of moths in an oviposition can will produce a compact mass. The spaces between the moths in this mass are very small, but not fixed. From the pressure of many other moths the female moths under such conditions receive a stimulus through the tip of the abdomen, and are thus induced to maximum egg deposition.

Oviposition cans of the size used at this Station (6 by 3 inches in diameter) give best results when they contain 10,000 to 15,000 moths. Moths live for about three days under crowded conditions, deposit most of their eggs during the first 24 hours, and at the end of the third 24-hour period are discarded and burned.

When oviposition cans contain enough moths (10,000 to 15,000) to form a compact mass one-fourth inch to three-fourths inch in

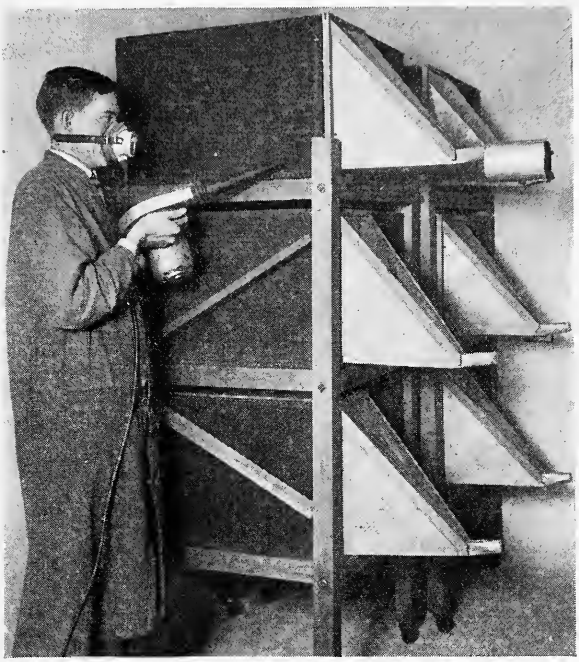

FIGURE 141. Showing process of removing moths from grain moth breeding unit. Oviposition can attached to end of funnel is removed and placed in an incubator, when the desired number of moths are obtained.

depth, a great deal of moisture collects on the trays under the cans, providing the humidity is 70 per cent or above, and the amount of moisture increases proportionately with a rise in humidity. Moisture under such conditions, will increase to such an extent as to hamper greatly the collection of eggs from the trays and cans. Much of the excess moisture that would be absorbed by the atmosphere were the relative humidity 60 per cent or below, causes the mass of moths to become soggy and moldy; likewise, it condenses on the

inside of the can and moths perish in it. The final result is a loss of most of the moths before maximum egg deposition takes place and a loss of a large portion of the eggs deposited as they become bunched or stuck to the moths and the inside of the oviposition cans.

Many of the male moths escape through the 30-mesh netting in the bottom of the oviposition cans, which is large enough for the exit of the smallest of them, but too small for any of the females to pass through.

SEPARATION OF EGGS WITH SIEVES AND ELIMINATION OF MITES

Although most of the eggs are deposited in the mass of moths in an oviposition can, a few are forced through the 30-mesh netting onto the trays. At the end of 24 hours the trays are cleaned into an 80-mesh sieve and the oviposition cans are shaken and tapped to extract the loose eggs. The nozzle of a 2-inch hose attached to an electric vacuum cleaner played on the underside of the sieve will suck out the dust, hairs, and scales. When mites are present, the suction draws and crushes them against the sieve, thus clogging the mesh; for which reason a change in the method of handling was found desirable. The dust, hairs and scales from the sieve, were then removed by tapping the sieve gently instead of by sucking. Eggs, mites and whatever foreign material remains in the sieve are then transferred three or four times from one fairly rough cardboard to another. This treatment removes all foreign matter and most of the mites from the eggs, which readily bounce and roll off the cardboards while all foreign material, including mites, remains.

Moths extracted from cages heavily infested with mites will always carry many of them into the oviposition cans. However, most of the mites have a tendency to loosen their hold on the moths during the first 24 hours and to drop through the wire mesh onto the trays, or are easily shaken from the mass of moths at the termination of the first period of egg deposition. The second period finds fewer mites and the third practically none.

Several grades of sieves were experimented with when endeavoring to find a means of separating moth eggs from foreign material such as dust, scales, hairs, mites and mite eggs. A 70-mesh sieve was tried, but the openings of the mesh were too large, allowing grain moth eggs to pass through endwise under vacuum suction. However, this mesh answered the purpose very well when the sieve was tapped, for then the eggs would not pass through. Mite eggs are very nearly spherical, having a diameter equal to the transverse diameter of the grain moth egg through its widest point (about .25 mm.). A mesh retaining Sitotroga eggs would also retain mite eggs or a mesh allowing Sitotroga eggs to pass through would also allow mite eggs to pass through. Therefore, an 80-mesh sieve was chosen, as it allowed neither species of eggs to pass through, either

by tapping or under suction. Passing the material over semi-rough cardboards to remove mites will also assist in removing some of the mite eggs.

CAUSE OF INFERTILE EGGS

Commencing February 1, 1931, and continuing until February 10, one of our rooms was inoculated with grain moth eggs. Each of the 18 cages received 50,000 eggs, none of which were more than 24 hours developed when placed in the cages. The first moths appeared 24 days after the first cage was inoculated. The average length of the first brood life cycle was 23 days, some of the cages having produced moths in 22 days.

On examination of the eggs from all of the cages one week after inoculation for percentage of hatch, it was found that on an average only 30 to 50 per cent had hatched, when there should have been an average of 80 to 90 per cent. It was at first thought that the eggs had dried out because the humidity in the incubator in which the grain moth oviposition units were retained during egg deposition was quite low, only 35 per cent, when it should have averaged from 60 to 80 per cent. However, after correcting this trouble, grain moth eggs procured in a 70 per cent humid atmosphere still continued to give a low percentage of emergence.

Shellac was considered as a probable cause of the trouble, so cards on which fresh grain moth eggs were fastened with shellac, and cards of eggs without adhesive material were placed together in a tray of wheat in the moth rearing room. Both groups of cards gave low percentages of hatch, the cards with shellac having a slightly lower hatch than those without.

A third possibility was injury to the eggs while collecting them. Thus the eggs that had dropped through the 30-mesh netting on the bottom of the moth egg deposition units were placed on cards beside eggs shaken from egg deposition units, for comparison of hatch. There was very little difference in the percentage of hatch between the two groups, the margin being in favor of the eggs shaken from the units.

Fresh wheat that had not been in the moth rearing room was placed in an 80° F. incubator, having a 70 per cent humid atmosphere kept in constant circulation by a 6-inch fan. After the temperature of the grain became equalized with that in the incubator, cards of grain moth eggs were placed in it. These egg cards gave a 25 per cent better hatch than another lot, hatched as a check in the moth room. An effort was then made to determine if the home-made humidifier, which had replaced the Braemer humidifier used during the first season of moth production, was supplying sufficient humidity. The Braemer humidifier was returned to the moth room and put in operation. After 24 hours fresh grain moth eggs were placed in the cages. These eggs gave the lowest percentage of

hatch of any, only 5 to 10 per cent. The percentage of humidity within the cages was taken and found to be but a few degrees below that in the room and the room humidity had averaged 73 per cent.

During the season of 1930 the moths, as previously stated, were extracted from the cages by a draft of air from a vacuum cleaner. The season of 1931 saw the replacement of this piece of apparatus with a portable blower that gave a very strong blast of air, thus facilitating the collection of the moths. It was thought that the moths were injured while being blown from the trays, possibly to such an extent as to inhibit mating. If this were true, many of the females would deposit unfertilized eggs. It is interesting to note here that although unfertilized eggs are of no value for re-inoculation purposes, they are just as good for Trichogramma parasitism as fertile eggs and the parasite develops normally within them.

Moths were collected both with and without the use of the blower. The eggs deposited by these moths gave equal percentages of hatch, 5 per cent. This result eliminated the blower as a detrimental factor in the investigation.

It was noticed, during the period over which these experiments were being conducted, that the eggs obtained from moths collected at intervals of five days gave a much higher percentage of hatch (60 to 75 per cent) than eggs from moths extracted at shorter intervals. This suggested that the female moths were not being fertilized if extracted from the rearing cages too soon after emerging. Moths were then extracted at intervals of from 1 to 10 days and the percentage of hatch recorded for the eggs obtained from these collections. It was found that when moths are extracted every day there are evidently many of them that do not mate before being blown into egg deposition units. As the chance of mating after getting into the units is greatly reduced, because of the way they are massed, a great number of the eggs deposited are sterile.

The results suggest that the longer the interval, up to one week, between moth extractions, the greater the chance will be of the females being fertilized, and thus a higher percentage of viable eggs obtained. However, this rule would only have to be followed when the egg production is to be used for reinoculation purposes. (Table 1.)

Table 1. Effect of Different Periods Between Moth Collections on Fertility of the Eggs Obtained from the Moths

Time since previous extraction of moths	Per cent hatch, eggs
6 hours	3
1 day	15
2 days	10
5 "	75
7 "	85

ADHESIVE MATERIALS FOR CARDS

Following the general procedure of Flanders (l. c.) the eggs are fastened to cards (Figure 146) with shellac. However, in order to learn whether shellac or other materials had any deleterious effect, a number of substances including shellac were tested with this in view. Gum arabic, glue, shellac, flour paste, wax, and even clear water were some of the materials tried, but of all these shellac has proven most satisfactory. The limiting factor in the ideal of adhesive substance is that which rain will not wash from the cards in the field. Gum arabic and flour paste, although excellent materials as stickers and non-toxic, are soluble in water and are of little value in holding eggs on cards during wet weather. Glue injures the eggs and wax covers them with a film impenetrable to the oviposition of the parasite. Not only must the potential damage resulting from rain be considered, but also the effect of the adhesive material on the embryo of the egg and on the parasite. Shellac, from all indications, has no ill effect on the developing embryo of Angoumois grain moth eggs, providing the eggs are pasted on cards within 24 hours after deposition, or immediately after removing them from a refrigerator where development has been arrested. Experiments (Table 2) show 90 per cent hatch from eggs shellacked on cards after 24 hours' development, 78 per cent hatch after two days; 72 per cent hatch after three days and 63 per cent hatch after four days' development. It is fair to assume that the farther along in their development the grain moth eggs are at the time they are shellacked to cards, the greater the mortality will be. This holds true only for eggs whose development has not been arrested by refrigeration.

The use of shellac to adhere eggs to cards for parasitism by Trichogramma involves another problem, that is to allow sufficient time to elapse between the shellacking of eggs on the cards and the exposure to the parasite. Alcohol, in which the shellac is dissolved, is detrimental to Trichogramma, and if it has not completely evaporated from the egg cards when they are exposed in the oviposition units, most of the parasites will die within a very short time and the majority of the grain moth eggs will remain unparasitized. Egg cards should therefore be allowed to dry for 30 minutes to an hour before placing with Trichogramma. Fifteen to 20 minutes were found to be insufficient.

TABLE 2. EFFECT OF SHELLAC ON DEVELOPING GRAIN MOTH EGGS

Days developed before fastening to cards	Per cent hatch
½	95
1	90
2	78
3	72
4	63

Incubation temperature 83° F., humidity 71 per cent.

EFFECT OF REFRIGERATION ON EGGS FOR INOCULATION OR PARASITISM

One of the outstanding problems to be dealt with in mass production of Angoumois grain moth eggs is the refrigeration of them either for Trichogramma parasitism or future wheat inoculation. It is practically impossible to hold grain moth eggs in cold storage[1] at temperatures below that at which embryonic development takes place and have them fit for wheat inoculation one week after being placed in refrigeration. If held at temperatures ranging from 40° F. to 46° F. (at which temperature development is practically arrested, but above which development takes place) for 24 to 36 hours, 90 per cent of the eggs will hatch when removed to higher temperatures; but, after two days of refrigeration less than 75 per cent will hatch, after three days 50 per cent, after four days 40 per cent and after a week or ten days only a very small percentage. Table 3 gives the results of grain moth egg hatch after refrigeration at 38° F. and 60 per cent humidity.

For inoculating wheat, fresh eggs or eggs that have been refrigerated not more than 36 hours should be used; otherwise, a much longer time will be required to build up grain moth populations because of a smaller initial brood.

For Trichogramma the eggs do not have to be fresh. If eggs are refrigerated after deposition they may be held for two to three weeks and still be in fair condition for parasitism (Table 4); but, if held longer than three weeks, only a small percentage will produce Trichogramma after being parasitized. These results were obtained from eggs held at 40° F. and 70 per cent humidity. At higher temperatures it may be possible to hold eggs for a much longer time and have them fit for parasitism. Humidity is most important as eggs will dry out and collapse in low humidity, thus shortening the length of time in which eggs may be refrigerated at any temperature. Partially developed grain moth eggs are more subject to killing by refrigeration (Table 3) than are those undeveloped.

TABLE 3. MORTALITY OF UNDEVELOPED GRAIN MOTH EGGS. REFRIGERATED AT 38° F. AND 60 PER CENT HUMIDITY

Number days refrigerated	Per cent hatch
0	100
1	90
2⅔	52
3	35
4	28
5	25
6	19
7	15
8	9
9	8
10	5.2
11	4.2
12	3.1

[1] Our storages are electric refrigerators.

TABLE 4. REFRIGERATION OF UNPARASITIZED GRAIN MOTH EGGS TO BE RETAINED FOR PARASITISM

Temperature 40° F.
Humidity 70 per cent

Number days refrigerated	Per cent turgid eggs
11	80
13	75
17	75
21	50
27	25

TABLE 5. MORTALITY OF PARTIALLY DEVELOPED GRAIN MOTH EGGS IN REFRIGERATION AT 43° F. AND 83 PER CENT HUMIDITY

Number days refrigerated	Per cent hatch
1 day pre-refrigeration development	
1	69
2	64
3	60
4	58
5	55
2 days pre-refrigeration development	
1	54
2	54
3	53
4	53
5	51
3 days pre-refrigeration development	
1	49
2	48
3	43
4	43
5	41
4 days pre-refrigeration development	
1	45
2	44
3	43
4	40
5	39

EFFECT OF HIGH TEMPERATURES ON EGGS USED FOR INOCULATION

While inoculating our grain moth cages during February and March, 1932, it was noticed that only a small percentage of the fresh grain moth eggs used for the purpose hatched. The greater majority of them collapsed and dried up within 48 hours after being placed in the trays, without evidence of embryonic development. Because of a similar experience while inoculating for moth production in 1931, all factors investigated at that time as possibly bearing on the subject were eliminated previous to collection of moths (and subsequently eggs) for inoculation purposes in 1932. However, the

fact remained that only 5 to 50 per cent of the grain moth eggs were producing larvae. It was thought that possibly the temperature was too high in the room in which the grain moths were reared, resulting in their infertility. Working from this angle a check-up on the room temperature was conducted and found to be averaging about 90° F. with a humidity of 70 to 80 per cent. This condition had existed during the greater part of the moth production period. The temperature was thereupon reduced to 80° F. and the humidity to 70 to 75 per cent. The eggs obtained from the moths reared under these conditions for an interval of two weeks gave a high percentage of emergence, 80 to 95 per cent, which indicated that a temperature of 90° F. and above with a high relative humidity probably would be detrimental to grain moth fertility.

DEPLETION OF WHEAT BY GRAIN MOTHS

The amount of wheat consumed by the grain moths during three seasons of rearing operations was determined in two ways: By weighing a definite amount of the grain before and after rearing, and by opening the kernels to determine the percentage that produced one or more moths. At the close of the 1930 and 1931 seasons the latter method was resorted to entirely while at the close of the 1932 season both methods were used in combination. The number of kernels producing one or more moths during the season of 1930, a duration of 11 months, was 73 per cent. This percentage was accepted as an average of 216 separate calculations of as many tablespoonfuls of wheat taken from various parts of the 18 moth rearing cages, comprising 12 trays each. At the close of the 1931 season of a little over nine months, the number of kernels having produced moths was determined to be 75 per cent. The method of procedure in arriving at this determination was similar to that in 1930; however, the number of calculations was less than the previous season. Although the amount of wheat by weight consumed in 1930 and 1931 was not computed, the weight of wheat in each tray was standardized at 4½ pounds at the start of each season's rearing.

In 1932 the amount of grain per tray was reduced to 3½ pounds on the assumption that the less the depth of wheat in each tray the more accessible the kernels at the bottom of the trays would become to the grain moth larvae and, therefore, increase moth production. The total number of trays receiving 3½ pounds of wheat each was 234 and the combined weight of wheat in these trays was 819 pounds. The rearing operations lasted exactly six months. At the end of this time the weight of grain in a large number of trays was determined and it was found that an average of 1¼ pounds per tray remained. Subtracting this figure from 3½ pounds, 2¼ pounds is obtained, the amount consumed. Multiplying 2¼ pounds

by 234 trays gives a total reduction of wheat of 526½ pounds, or 64.2 per cent of the total amount of wheat (by weight) at the start. The amount of wheat not consumed was 292½ pounds.

A small quantity of wheat was taken from each of a large number of trays and the kernels opened. It was found that 92 per cent of all the kernels had produced grain moths and of this percentage 72.8 per cent had produced two grain moths per kernel (all a wheat kernel is capable of with few exceptions) leaving nothing but empty shells. The remaining 27.2 per cent of the 92 per cent infested kernels had produced one grain moth per kernel and were, with few exceptions, capable of providing food sufficient for the development of one more grain moth per kernel. The number of grain moth eggs procured from the moths reared on 819 pounds of wheat in 1932 was 39, 440,000. In 1930 and 1931 from 972 pounds of wheat each season, 14,912,000 eggs were collected in 1930, and 22,957,400 eggs in 1931.

The above figures serve to demonstrate the efficiency of a smaller amount of grain per tray in 1932 over that of a larger amount the previous seasons. However, this does not tell the whole story, as the number of eggs used to inoculate the grain and the time required for inoculation have some bearing on the results obtained. In 1932 a greater number of eggs were used for inoculating purposes over a shorter period of time than in 1930 and 1931. This would ultimately mean a greater moth yield and thus a larger number of eggs procured over a shorter period of time, and finally a more rapid and thorough depletion of the grain.

QUANTITY PRODUCTION OF GRAIN MOTHS

1930

From March 20, when extraction commenced, until the middle of November, when the units were cleaned out, it was estimated that 18,000,000 eggs were produced. Extractions of moths were not as frequent towards the end of the period because parasite production did not demand it. From 41,000 to 441,000 moths were taken from the cages at one time, as estimated by counting and weighing. A greater part of the eggs were used for parasitism and about 50 per cent of those parasitized were liberated. Others were used for refrigeration, hibernation, and other experiments.

1931 and 1932

During these years there was a considerable increase in the number of eggs obtained because of increased facilities, including an additional room and six more units than were used in 1930. As in 1930, a considerable number of eggs were used for experiment. In 1932, the amount of wheat used was considerably reduced, owing mainly to the discovery that 27 per cent of the wheat used in 1930

and 1931 remained uninfested at the end of the season. The increased availability due to more room between the trays enabled the moths to infest successfully more than 90 per cent of the kernels in 1932. Tables 6 and 7 give statistics of production in these years.

TABLE 6. GRAIN MOTH EGG PRODUCTION

1931		1932	
January	1,896,000	February	1,650,000
February	1,700,000	March	3,050,000
March	750,000	April	1,780,000
April	690,000	May	7,284,000
May	5,790,000	June	7,544,000
June	7,530,000	July	9,544,000
July	3,682,000	August	6,368,000
August	5,590,000	September	1,752,000
September	1,070,000		

TABLE 7. RESULTS OF THREE YEARS OF GRAIN MOTH REARING

Season	Duration of production	Wheat used for rearing, bu.	Per cent of kernels that produced moths	Seasonal egg production
1930	11	16	73	18,640,000
1931	9	22.9	75	28,698,000
1932	8	17.8	92	38,972,000

ENEMIES OF THE GRAIN MOTH AND METHODS OF CONTROL

Mites of several families, grain beetles, ants, and the larval parasite (*Dibrachys boucheanus* Ratz.) are the principal enemies of the grain moth that have been encountered.

The first to appear were mites of the superfamily *Parasitoidea,* formerly known as gamasids. These were present in original stocks of moths and precautions were taken to exclude them from breeding rooms by removal of their eggs with screens, and by hand picking under a microscope. In spite of all precautions they reappeared in the breeding rooms by the middle of April. Control experiments (Tables 8 and 9) were begun from which it was learned that they may be kept in check by heating the rooms and lowering the humidity to 10 to 15 per cent. Perhaps the most important factor in their control lies in frequent removal of dead moths from the cages, since the mites feed and multiply mainly on dead indi-

viduals. Our breeding system fortunately allows for removal of all moths whenever desired. It was also learned that mites of this group pass from the oviposition can after the first 24 hours. Those coming through with the eggs may be killed by refrigerating five to six days at 38° F.

TABLE 8. HEATING EXPERIMENTS TO DESTROY MITES

Number	Time of exposure minutes	Temperatures	Depth of wheat	Per cent killed
1	90	105.8–111.2.	1½ in.	0
2	150	105.8–111.2	1½ in.	90
3	120	105.8–113	1½ in.	100
4	90	105.8–111.2	¾ in.	0
5	120	105.8–113	¾ in.	100
6	150	105.8–111.2	¾ in.	100

Moths not killed in these experiments. Humidity 57 per cent throughout.

TABLE 9. TESTS IN HEATING THE GRAIN MOTH ROOM TO DESTROY MITES WITHOUT DESTROYING THE MOTHS

Time exposed hours	Temperatures °F.	Humidity	Kill of mites
2	110–113	60	None in trays. Few in open compartment.
15	104	60	None in trays. Few in open compartment.
6½	106–122	20	None in trays. 100 per cent in open compartments.
17	97–118 (av. 102)	20	100 per cent. 95 per cent of moths also died.

It appears possible from the above tables to kill mites of the superfamily *Parasitoidea* without killing the moths, though it is difficult to secure control by this means alone without reducing the moth population too much. It is evident, however, that higher temperatures such as were employed to sterilize the cages in the beginning would be fatal and would probably remove all danger of their introduction in the wheat or grain used to stock the cages.

Tyroglyphidae (flour and cheese mites) appeared in some of our preliminary breeding cages, but have never been serious.

Book lice *(Corrodentia)* appeared in some numbers for a while, but disappeared after the first year and few have been seen since.

During the seasons of 1931 and 1932, *Laemophlaeus pusillus* multiplied to such an extent that it became a very serious problem,

especially during the late winter and early spring of 1932. This is a very small beetle that is able to slip into the cages and goes easily from one to another. The 1931 infestation was less severe and lasted for a much shorter period than that of 1932. *Cephalonomia waterstoni,* Gahan[1] a parasite of *pusillus* larvae, was discovered to be actively engaged in checking the 1931 beetle infestation shortly after the first beetles were observed on the outside of the moth cages. Where the parasite came from is not certain, but it succeeded in completely eradicating the colony of beetles and then, due to lack of food, disappeared almost entirely.

The heaviest infestation of *Laemophlaeus pusillus* occurred in 1932. The beetles and their larvae were so numerous during the winter and spring they attacked the grain moths and their eggs. Each time moths were extracted large numbers of beetles and larvae came with them. The larvae were exceptionally destructive in getting into the grain moth eggs and feeding on them. Not only did a day's collection of moth eggs contain a large number of beetle larvae, but it likewise contained many beetle eggs. These eggs were so much like the grain moth eggs in appearance, particularly in length and color, that it was impossible to separate them from the grain moth eggs. Because of this limitation *pusillus* eggs were fastened with grain moth eggs to cardboard disks and exposed with the latter to Trichogramma for parasitism. The beetle eggs were not parasitized and on hatching, their larvae attacked both parasitized and unparasitized moth eggs. *Pusillus* larvae were also observed pulling Trichogramma pupae from grain moth eggs and devouring them.

As during 1931, the parasite *Cephalonomia waterstoni* appeared and multiplied in unbelievable numbers. Its attack on *pusillus* was not appreciated for some time because of large numbers of the host, but it finally succeeded in bringing the beetle under control after several months. *Cephalonomia* adults possess an irritating sting with which they paralyze their host larvae. After stinging and feeding on the larvae the parasite carries away its victim and buries it.

The confused flour beetle, *Tribolium confusum,* became numerous in 1932 in one unit and destroyed much wheat. Sterilization by heat before dismantling and before inoculation, has eradicated them and they have not reappeared. Its eradication is described in detail on page 707.

Ants began to appear shortly after the rooms were filled with wheat in 1930. They succeeded in penetrating the cages and began to attack the moths. Tanglefoot on the legs of the carriages was ineffective in preventing damage. Oil repellents supplied by a large oil company were then found effective in keeping them out of the room and these were used whenever ants reappeared.

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[1] Family Bethylidae.

The grain moth parasite, *Dibrachys boucheanus* Ratz., was troublesome in 1932 during the summer months. However, it did not become abundant until late summer and was destroyed when the rooms were heated on dismantling the cages and removing the wheat.

So far, rooms have not become infested with *Pediculoides ventricosus,* an enemy of the grain moth commonly reported by other workers in this field. Care was taken to obtain grain from small grain houses in the outskirts of town, instead of large plants where there is more likelihood of obtaining pests of this kind. This safeguard, together with preheating the entire room before inoculation, probably prevented *Pediculoides* from becoming established. At any rate we consider ourselves fortunate in not being troubled by this most important enemy of the grain moth.

An example of the heat treating methods employed follows.

HEATING BEFORE DISMANTLING MOTH ROOM

When dismantling of the six cages in the smaller moth room commenced on April 22, 1932, it was found that the grain beetle, *Tribolium confusum,* had increased to enormous numbers. There were several other grain and moth pests present in sufficient numbers with *Tribolium* to warrant treating the room before removing the used grain. Therefore, on April 22 the room was heated for five hours by means of three electric heaters and one gas stove to an average temperature of 159° F., with a minimum of 134° and a maximum of 172°. At the end of this period it was found that the moths and beetles killed were those that had been flying around the room and those in the extracting compartment of the cages. On April 24 the trays were removed from the cages and piled on the floor of the room in such a manner as to allow the heat to penetrate all parts. The average temperature during this second heating period was 180° F. for seven hours, with a minimum of 141° and a maximum of 201°. The average temperature for the last five of these seven hours was 189°. Surprising as it may seem, there were still a number of live beetles in the center of the trays after being subjected to this intense heat. The centers of the trays were not completely exposed when piled on the floor, although enough space was left between them for air circulation. Where the trays were completely exposed, the heat penetrated sufficiently to kill all of the beetles.

The trays were emptied of their grain on April 28 and the room heated for the third time to destroy all beetles that had escaped the first and second heating periods. This time the room was heated for three hours to an average temperature of 161° F., with a minimum of 113° and a maximum of 188°. As there were still a few beetles alive at the end of this three-hour period, the room was

heated again the following day for seven hours at an average temperature of 192° F. The minimum temperature was 158° and the maximum 210°.

Upon examining the room April 30, several live beetles, moths and book-lice were found on the floor. These had undoubtedly been concealed in crevices where they were protected from the full force of the heat. The room was again heated for a period of two days without reducing the heat overnight. The average temperature for the two days was 149° F., the minimum average was 132° and the maximum average 161°.

When the room cooled all of the cages and trays were vacuumed and washed, and on May 4 were restocked with fresh wheat. For three days thereafter the room was heated to an average temperature of 184° F., a minimum temperature of 134° and a maximum of 203°. This heating was for the purpose of killing all potential grain pests.

On May 9, the cages were inoculated with 60,000 grain moth eggs each.

GENERAL PROCEDURE FOR MOTH PRODUCTION

In order to summarize and condense the information presented in the preceding pages, the following plan of procedure is given. This is the method used to obtain eggs in large quantities as finally adopted after two years' experience. See Figures 139 to 141 for apparatus used, also Figure 145.

PROCEDURE

(1) Sterilize grain (wheat preferably) for eight-hour periods at 150° F. to 210° F.

(2) Humidify grain at 70 per cent relative humidity for a few days to a week before inoculating.

(3) Inoculate at the rate of 50,000 grain moth eggs to one bushel of grain.

(4) Allow third generation of grain moths to reach its peak before removing many moths.

(5) Extract moths every second day, or three times a week.

(6) Hold extracted moths at 80° F. and 50 to 60 per cent relative humidity for three successive 24-hour periods to obtain maximum egg deposition.

(7) At the termination of each 24-hour egg deposition period, shake oviposition units gently to remove eggs.

(8) Refrigerate eggs at 38° F. and not less than 60 per cent relative humidity.

(9) Eggs unparasitized should not be held in refrigeration longer than two weeks if they are to be used for parasitism and not more than two days if they are to be used for reinoculation purposes.

STUDIES ON TRICHOGRAMMA EGG PARASITES

Egg parasites of the genus Trichogramma have been known to occur in the United States since 1856. They attack many important hosts such as the Oriental fruit moth, the cotton worm, codling moth and others. Their usefulness has long been recognized. It was not, however, until 1927 when Flanders perfected his breeding method that they began to be studied seriously in this country as a possible measure of control. Since then many plants for rearing

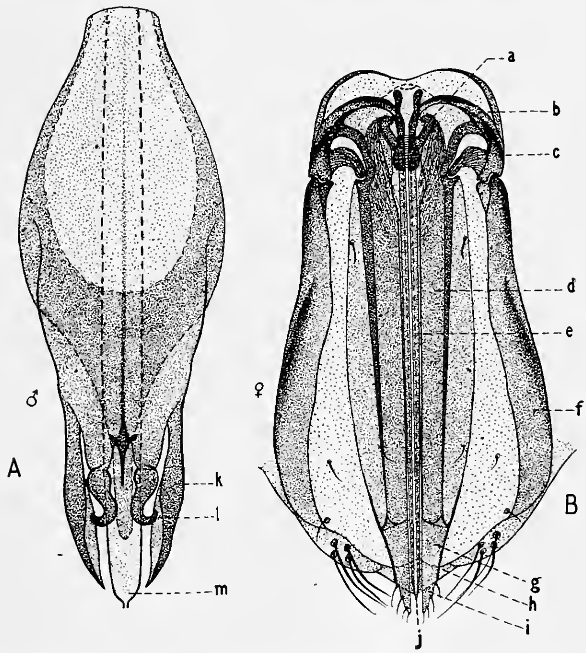

FIGURE 142. Ventral aspect of genitalia, male (A) and female (B) Trichogramma. Comparison of the different strains showed no differences in these structures. a, inner valve bases; b, stylet bases (ventral valve); c, valvifer (VIII sternite); d, proximal part of dorsal valve; e, inner valve; f, IX sternite; g, distal portion of dorsal valve; h, X tergite; i, tip of dorsal valve (IX sternite); j, tip of ventral valve (VIII sternite); k, modified harpes; l, inner gonopods; m, penis.

them have been put into operation, including the commercial plant of Dr. A. W. Morrill, of Los Angeles, where they are produced in great quantities.

From a commercial standpoint, it is apparently possible to rear the insects profitably for Dr. Morrill quoted them as low as 12 cents per thousand in 1932.

Much discussion of the true value of Trichogramma has, however, taken place in entomological circles, and the difficulty of obtain-

ing proper checks for field experiments has probably caused many to abandon their studies because of their apparent futility. Smith and Flanders[1] have criticized the general idea of successful control by liberating Trichogramma. However, there seems to be so much to be learned concerning it that even if these parasites are not effective in bringing about desired reductions in infestations, studies of

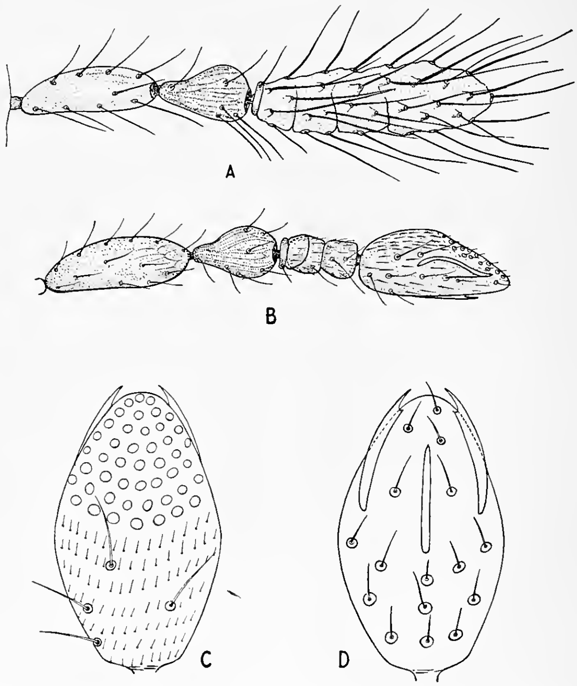

FIGURE 143. Structural details of the antenna of Trichogramma. A, male; B, female; C and D, ventral and dorsal aspects of the terminal segment of the female antenna.

field liberations combined with laboratory investigations will undoubtedly yield much of value to this phase of entomology.

The species names for the parasite most frequently occurring in American literature are *Trichogramma minutum* and *Trichogramma pretiosa*. However, there are a number of others used in reports on this parasite during the latter part of the last century. Noteworthy among them are: *T. odontatae, T. orgyiae, T. nanum, T. fraterna, T. intermedium, T. minutissimum,* and others. These, however, have been completely displaced by *minutum* and *pretiosa*.

[1] Jour. Econ. Ent., 24: 666-672. 1931.

Whether there was any basis for the gradual adoption of the two names is doubtful, as later the name *minutum* practically took precedence over all others, including *pretiosa,* and is generally accepted to be the proper name for the parasite in practically all recorded cases of parasitism in this country. Entomologists can hardly be blamed for this confusion since structurally, all American forms of the genus appear to be identical. There was in all probability some basis for the distinction between *minutum* and *pretiosa* in the color characters of the two. C. V. Riley, in his Third Annual Report for the State of Missouri, 1871, gives a description of a species of Trichogramma that he tentatively called *minutum,* and stated that it is a "little dark-colored four-winged fly." In the Canadian Entomologist for 1879-80, Riley describes another species of Trichogramma as being yellow and affixed to it the species name *pretiosa.* As will appear later, these two forms, although identical in structure, show important differences which we believe are distinctive enough to warrant retention of Riley's original names. The form most commonly encountered in New England may be regarded, therefore, as *Trichogramma pretiosa.* Some of the structural features are shown in Figures 142 and 143.

A large number of collections of parasitized eggs of the Oriental fruit moth were made in 1931 and 1932. All the 1931 recoveries except one were the "yellow" species *(pretiosa),* while only a few collected from the field in 1932 were the "dark" form. Table 10 shows the results of some of the collections made in 1931.

The form most commonly encountered in Connecticut may be regarded, therefore, as *T. pretiosa.* Table 10 shows results of field collections to determine whether *pretiosa* or *minutum* predominate in Connecticut.

HABITS AND LIFE HISTORY

The life history of Trichogramma is similar in field and laboratory. The life cycle may be long or short in the field, due to weather conditions, or it may be lengthened or shortened as desired in the laboratory by regulation of temperature and humidity. The characteristic work of the parasite is the same for all of its hosts. Its entire life cycle is passed in the egg of its host and on emerging the adult parasite is ready to attack others hosts' eggs and thus continue its species.

The adult parasite is a small Hymenopterous wasp with a wing spread of about one-fiftieth of an inch. It has two pairs of wings, the fore pair being fan-shaped with a fringe of fine hairs around the margin and lines of hairs on their upper surface. The hind pair of wings is lance shaped and is fringed and lined with hairs as are the fore-wings. The adults vary in color from light lemon yellow to black through the range of the several species investigated. The sexes are distinguished principally by differences in the antennae

and in the shape of the abdomen. Color is of some assistance. Males of *minutum* have a dark abdomen while the abdomen of the female is entirely lemon yellow. In refrigeration or when reared at low temperatures, the dark color of the males is more intense than when reared constantly at high temperatures. The females are slightly darker at low than at high temperatures, but never so dark

Table 10. Trichogramma Recoveries in Connecticut, 1931

Orchard where recovery was made	Date	Species, color
L. C. Root & Son, Farmington	June 27	yellow (*pretiosa*)
	July 16	"
	Aug. 3	"
	Aug. 21	"
	Sept. 11	"
W. F. Platt, Milford	July 18	yellow "
Kneuer Orchards, Guilford	July 16	yellow "
	Aug. 11	"
Avery Orchards, Yantic	July 16	yellow
	Aug. 6	"
Conn. State College, Storrs	July 16	yellow "
	Aug. 6	"
	Aug. 21	"
Pero Brothers, North Manchester	June 15	yellow "
	June 27	"
	July 16	"
	Aug. 21	"
Station Farm, Mount Carmel	July 17	yellow "
	July 23	"
	July 27	"
	July 30	"
	Aug. 18	"
	Aug. 28	"
	Sept. 1	"
	Sept. 2	"
Starr Orchard, Fairfield	July 7	yellow "
Experiment Station, New Haven	Jan. 12	yellow "
	Feb. 25	"
	July 13	"
	July 20	dark (*minutum*)

as not to be distinguishable from the males. This distinguishing color characteristic holds true for the light species of Trichogramma only; the males and females of the dark species are both dark in color, making it difficult to separate the sexes without inspection of the antennae.

Female Trichogramma oviposit in host eggs at random. Their eggs hatch in a few hours and the larva destroys the embryo of the host egg. The parasite larval stage at 80° F. is from two to three

days in length; at the end of this time the larva covers the inside of the host egg with a film which soon turns black. The black color, readily seen through the chorion of the host egg, indicates that the parasite has entered the prepupa stage. Four and one-half days later the adult parasite emerges, completing its life cycle in 6½ to 7½ days at 80° F. and 70 per cent humidity. Trichogramma are sexually mature and positively phototropic upon emerging from the host egg. They may move about immediately on issuing, slowly at first and more rapidly as they become dry. Some remain, while drying for a short time on the host egg shell, while others move away in a few seconds. If any part of the pupa case remains on the posterior end of the abdomen, or if any particles of chorion adhere to the head or antennae the parasite cleans them off as soon as it is fully out of the egg. While drying, the parasite cleans its antennae and legs very often. It uses its metathoracic legs to clean and dry the wings, likewise to facilitate their expansion. While working on one fore-wing the other fore-wing is held perpendicular to the body. The wings are rubbed dry and open by both the metathoracic legs working in unison. The legs hold one wing at a time laterally to the body, the costal margin of the wing being in a ventral position. Both legs start at the base of the wing, one on either side, and move downward to the apical margin. This is repeated many times until the wing is completely open. The parasite may then move about a little, but soon stops and repeats the rubbing process until the wings are completely dry. They are then held folded over the abdomen, the right fore-wing overlapping the left. Sometimes they are carried in this manner, while again they may be raised and separated at an angle from the body. It is the usual thing for Trichogramma to have fully developed wings, but sometimes a number of them have vestigial wings. This has been especially noticed with Trichogramma that have been in refrigeration; the longer the period of refrigeration, the greater the percentage of parasites with undeveloped wings.

The number of Trichogramma per host egg varies considerably for its many hosts. Some hosts have but one or a few parasites per egg, others have many. The small sugar cane borer, *Diatraea saccharalis* Fab., is recorded as having as many as 10 Trichogramma per egg. There is usually only one parasite per Angoumois grain moth egg when the moths are reared in wheat (moths reared in wheat are smaller than those reared in corn and thus deposit a smaller egg), although two have been recorded emerging from a single egg. In such a case, they may be of the same or both sexes. In the field, fruit moth and currant sawfly eggs very often have two and three parasites per egg. When there is more than one Trichogramma per egg, they all issue through a single exit. The first parasite to issue chews through the chorion of the egg a hole large enough to squeeze through. It is soon followed by the remaining

parasite or parasites, as the case may be, all of which may enlarge the opening a little. In the laboratory from one-half to three-quarters of an hour is required from the time the parasite begins eating its way out of the egg until it emerges and its wings are expanded and dry. The parasite may mate in a very few minutes after emerging before its wings have expanded and dried; or it may not mate until dry and normally active.

In the field, weather conditions greatly determine the activity of the adult parasite. If the weather is cold and stormy, the parasite remains inactive, or on the leaves and branches of the trees. Humidity plays an important part in the longevity of the adult parasite. It will live longer at high than at low humidity both in the field and in the laboratory. Likewise, the mortality is greater at low humidity when the parasite is still within the host egg. It hibernates in an immature stage in native host eggs. In some of the southern states and in tropical countries, Trichogramma continuously perpetuates itself in host eggs present the year round. There are sections in the sugar cane belt where the adult parasite survives a short winter hidden away in trash and litter in the cane fields.

HOSTS ATTACKED

There are about 215 species of insects known to have Trichogramma as their egg parasite. Eggs of the currant sawfly, *Pteronidea ribesii,* have been found parasitized as early as May 9 at New Haven. Trichogramma species have also been recovered from canker worm eggs *(Alsophila pometaria* Harris) and the lace wing fly *(Chrysopa* sp.). It has been reported from the sugar cane borer *(Diatraea saccharalis* Fab.), the codling moth *(Carpocapsa pomonella),* the fall webworm, brown tail moth, and many other important pests.

MATING HABITS

Mating sometimes takes place as soon as the adults emerge and before they have thoroughly dried with normally expanded wings. As a rule, males emerge in greater numbers than females during the initial appearance of adults from the same stock of material. Both sexes remain on the cards for a short while if emergence takes place in darkness or in subdued light. Under such conditions, activity is at a minimum and mating is not so certain as in the presence of a moderate or strong light. When the amount of light is sufficient to stimulate adults to normal activity, a large number of males remain on the egg cards and mate with females as they appear. Others move around the parasite dishes in which they have emerged and mate readily with females with whom they come in contact. Both sexes are negatively geotropic and positively phototropic in all

the species investigated. However, there seems to be a more pronounced response to light by the forms of the yellow species *(pretiosa)* than by those of the dark species *(minutum)*, but both species may be concentrated with proper lighting so as to obtain the best results from a standpoint of fertility. In total darkness, the adults do not emerge to any extent from their host eggs for some time after they would have normally appeared had light been present. Exposing the parasitized host eggs to light after having held them in darkness beyond the parasites' minimum period of development will bring practically all of them forth in two to three hours. The advantage of this treatment is that it brings large numbers of individuals of the same age together and insures mating.

MAXIMUM GENERATIONS OF TRICHOGRAMMA OCCURRING IN CONNECTICUT IN INSECTARY IN 1931

The method employed in arriving at the maximum number of Trichogramma generations that would probably occur in Connecticut in one year, was adopted because of the limited amount of material on hand and the lack of help required for carrying on the investigation in greater detail.

Trichogramma parasitized grain moth eggs placed out-of-doors late in the fall of 1930 were observed from time to time throughout that winter and into the spring of 1931, for early emergence of the parasite. During late March and early April a few parasites that survived the winter emerged. On April 16, freshly emerged Trichogramma of the yellow species were exposed to grain moth eggs in an insectary on the Experiment Station grounds. Small watch-glasses similar to those used in the laboratory for mass production of the parasite were adopted as parasite oviposition units for insectary work.

The day on which the initial emergence of each successive generation took place, fresh grain moth eggs on small cardboard disks were exposed to the parasites. These eggs were watched closely to note the date of darkening and thereafter the appearance of the first individuals from the ensuing generation. The date of the initial emergence of the parasite did not always coincide with that date on which fresh grain moth eggs were exposed to the adults. Thirty-six per cent of the exposures were on the date of parasite initial emergence, while the remaining 64 per cent varied from one to four days from the date of emergence.

From April 16 to November 25 there were 15 full generations (Table 11). Grain moth eggs were not exposed to the 15th generation, as all of the eggs parasitized by the 14th generation did not hatch during November and December, 1931; many of them carried over until April, 1932. The period of parasite, larval, and prepupal development varied considerably, depending on the optimum tem-

perature during the time each generation was in the process of development. From April to June the difference for each successive generation was two days less than the preceding generation. During July and August larval and prepupal development was the same for the three generations occurring in each of the two months, with the exception of the third generation in August which required one additional day. The first generation in September had an equal period of larval and prepupal development with that of the third generation in August, while the second generation in September required one additional day. The October generation developed as rapidly through the larval and prepupal stage as did the second September generation. However, the larval and prepupal development of the 14th generation required 12 days, or seven days additional to those of the two previous generations.

Maximum adult emergence was not reached on the day the generations started to issue. The maximum abundance of adults occurred at varying dates after initial emergence. However, this peak of abundance for each generation shows a tendency to draw nearer to its initial emergence from the first to the midsummer generations, and from then on to the 15th generation there is an increase in the time required for each successive generation to reach its maximum abundance. The maximum abundance of the first generation occurred about the seventh day after the first emergence. The midsummer generations reached their maximum abundance on the first or not later than the second day of emergence; while the 14th and 15th generations required about eight to ten days respectively for the peak of maximum abundance to occur. (Table 11.)

There were but two successive generations having life cycles of equal length; these were the 10th and 11th, which required 10 days each for development. The 8th generation required the least number of days for development, July 23 to 31, or a period of eight days. The longest period of development was 33 days, October 23 to November 25, required by the 15th generation. The second generation came nearest to this with a period of 29 days of development, April 16 to May 16. The number of days of development decreased for each generation from the first to the midsummer generations. From then on the condition was reversed and the period of development gradually increased to the 15th generation.

Regarding the longevity of the adult Trichogramma (Table 11), there was a close correlation between length of life cycle and maximum survival of the adult parasite. A few of the first generation adults lived 21 days. The seventh and eighth generation adults, occurring in midsummer, survived the shortest length of time, five days each. The 14th generation adults survived longer than those of any other generation with the exception of the 15th, when taking into consideration the maximum number of survivals. Two males of the 14th generation remained alive after all the other individuals

had died. One of these males died the first week in February, while the second one lived until February 19, or approximately four months.

TABLE 11. RATE OF DEVELOPMENT AND LONGEVITY OF TRICHOGRAMMA IN INSECTARY, 1931

Parasites emerged	Parasites exposed with fresh eggs	Number days required for parasitized eggs to darken	Length of parasite life cycle	Longevity of adult parasites
April 16	April 16	8 days	29 days	21 days
May 16	May 16	6 "	14 "	11 "
June 1	June 4	4 "	13 "	11 "
June 18	June 19	4 "	11 "	13 "
June 30	July 1	3 "	12 "	12 "
July 13	July 13	3 "	10 "	7 "
July 23	July 23	3 "	8 "	5 "
July 31	Aug. 1	3 "	9 "	5 "
Aug. 10	Aug. 10	3 "	10 "	7 "
Aug. 20	Aug. 22	4 "	10 "	10 "
Sept. 2	Sept. 3	4 "	11 "	8 "
Sept. 14	Sept. 15	5 "	14 "	8 "
Sept. 29	Sept. 30	5 "	19 "	13 "
Oct. 19	Oct. 23	12 "	33 "	119 "
Nov. 25	No	No "	No "	35 "

Note—Yellow species of Trichogramma used, native to Connecticut; 15 generations in 1931, April 16 to November 25.

OVERWINTERING HABITS OF TRICHOGRAMMA (OUT-OF-DOORS)

Due to the fact that there are few Trichogramma in the spring in northern latitudes, it was generally considered that the parasite was unable to survive the cold winter weather and therefore came through year after year in extremely small numbers, probably only a fraction of 1 per cent. However, through our observations over the past three years, both in the field and in the laboratory, we are convinced the native species of Trichogramma is surprisingly hardy; in fact, so much so that it will complete its development in refrigeration at 49° and emerge. In all probability the relatively low percentage of peach moth egg parasitism during the month of June in Connecticut is directly due to the small number of Trichogramma present at that time. Theoretically as the season advances and host material becomes more abundant, the parasite multiplies at the expense of the host, resulting in a much higher parasitism during the latter part of the summer.

The yellow strains of the light species of Trichogramma are generally distributed throughout the colder regions, while the dark strains of an entirely different species are found in the warmer parts of the country. We have been unable to recover the dark species

of Trichogramma in Connecticut in any numbers, while numerous collections of the yellow species have been made. Therefore, only the yellow species was used in our overwintering experiments in the field, while both the dark and light species were used in refrigeration experiments in the laboratory. The results of the field experiments only will be discussed here.

Hibernation cages were constructed for the purpose of determining how Trichogramma passes the winter in Connecticut, in what stage or stages, and in about what numbers. There were four of these cages, 8 by 10½ by 9½ inches. The frames were made of three-fourths inch white pine and covered on the bottom and the top with one cross bright finish tin and three sides with 50-mesh wire screening. The door of each cage was of framed glass and was screwed into place with six 1¼ inch screws. All joints and crevices were filled with celluloid cement and finished off with paraffin. The inner face of the three sides was covered with a fine texture muslin fastened into place with paraffin. The frame work and tin were given one coat of asphaltum as a preservative. A layer of leaves was placed in the bottom of each cage to a depth of three inches. After the doors had been put in place and screwed tight, putty was used to make the connections water tight.

Adult Trichogramma of the yellow species were liberated in two of the cages, parasitized peach moth eggs in the third cage, and parasitized grain moth eggs in the fourth. After sealing, one cage containing adult parasites was placed in a sheltered location, while the second cage, containing adults was placed in a semi-exposed location. Both of the cages containing parasitized peach moth and grain moth eggs were placed together in an unexposed location. All of the cages were placed out-of-doors November 6.

On examining the cage containing parasitized grain moth eggs February 26, it was found that a few eggs had hatched. Some of the eggs were then removed to an 80° F. incubator and from these both male and female parasites emerged. To date, the eggs had been in the field 125 days. Again on March 26 another lot of eggs from the same cage were examined and some of them were hatched. The remainder of this lot produced a few males and females when subjected to 80° F. The eggs were now 140 days in the field.

On April 3 a third examination of the same cage revealed a similar condition as recorded for the previous examinations. A number of eggs had hatched and several adults were obtained from a few of the eggs at 80° F. All of the cages were examined on April 21. No living adult Trichogramma were found in either of the cages in which they had been liberated. The parasitized fruit moth eggs had not hatched nor did they when subjected to 80° F. The remainder of the parasitized grain moth eggs that successfully passed the winter had hatched, but none of the living adults could be located. Table 12.

Parasitized grain moth eggs fastened to cardboard disks with shellac and hung in peach trees in sealed wax-coated bags on October 23, 1930, produced male and female Trichogramma at 80° F. on March 17, 1931. On April 21, 1931, one female parasite issued from an egg card that had been sealed in a wax-coated bag and buried in a box of leaves under a peach tree October 23, 1930. Parasites had issued previous to this time from the same card, for the dead adults were found in the bag. One egg card was examined on December 4, 1930, and some of the eggs were found to have hatched. Several adults were still alive when the examination was made.

The results show that Trichogramma having a short life cycle at summer temperatures will develop very slowly at lower temperatures, during warm spells late in the fall and early in the spring. Likewise, development continues during the winter months whenever the temperature permits.

During the winter of 1931-32 a second investigation was conducted into the possibility of overwintering the yellow species of Trichogramma out-of-doors. This time watch-glasses similar to those used in mass production of the parasite were adapted as hibernation units, in place of the cages of the previous winter. Cards of parasitized grain moth eggs, parasitized on October 23, 1931, were inserted in the watch-glasses in a screened insectary, where they were retained throughout the experiment. A close watch was kept on the watch-glasses and on November 25 the first parasites to emerge were noted moving around on the under side of the egg cards. These adults were removed to empty watch-glasses, where they died 35 days later. A daily examination was made of the egg cards, from which the parasites emerged on November 25, for further emergence. At various times throughout the next five months, adult Trichogramma appeared on these cards, emergence taking place whenever the optimum developmental temperature for Trichogramma occurred for a number of successive days.

Nearly all the parasites emerging prior to the latter part of March died before April 1. However, a few that emerged in late March survived well into the month of April, and those to emerge in April died before April 26. On May 2, 1932, two adult Trichogramma emerged from the eggs parasitized on October 23, 1931. One was a male; the sex of the second was not determined. The undetermined adult lived but one day, while the male lived two days. Of the 40,000 parasitized October 23, 1932, 74.99994 per cent of the total emergence of 75 per cent emerged between November 25, 1931, and April 26, 1932. The remaining .00006 per cent, or two parasitized eggs, emerged May 2, 1932. The maximum survival, therefore, for Trichogramma in grain moth eggs in the Station insectary during the winter of 1931-32 was six months and nine days. The percentage of maximum survival for the same period was .00006 per cent.

Regarding the longevity of adult Trichogramma some interesting information was obtained during the winter of 1931-32. Two male adults that emerged on October 19 in our insectary remained alive and active until midwinter, 1932. One of these died the first week in February, and the second one died February 19, approximately four months from the date of emergence. This was quite unusual as the longest longevity previously recorded at this Station was 35 days. A few adults that emerged in early December lived until early January. However, weather conditions must be taken into consideration in connection with the above facts, and also in winter emergence of Trichogramma. The fall of 1931 and winter of 1932 were abnormal for this locality. Exceptionally mild weather prevailed throughout the two seasons, and normal winter weather did not occur until after February 1; even then it was not severe. These factors naturally account for a more rapid development of the parasite during a time when it should be hibernating, or when development would normally be at a minimum. Extremely low temperatures for varying lengths of time are detrimental to the adult parasite. At a constant temperature of 38° F. for 12 days, all Trichogramma perished. However, at fluctuating temperatures, especially when above freezing, the longevity of adult Trichogramma is considerably lengthened.

Table 12. Mortality of Trichogramma in Hibernation Cages, Winter of 1930-31

Cage	Stage in which parasite over-wintered	Date cage was placed	Where cage was placed	Exposed or sheltered	Results
1	Adult	November 6	In lee of brick building	Sheltered from north-west winds	All dead April 1
2	Adult	November 6	On east side of a wire fence	Semi-exposed to northwest winds	All dead April 1
3	Pupa stage in peach moth eggs	November 6	On east side of a wire fence	Completely exposed	All dead April 1
4	Pupa stage in grain moth eggs	November 6	On east side of a wire fence	Completely exposed	2 males 4 females emerged April 3

Note: The parasitized eggs used for adult emergence and for over-wintering were one week old November 6.

In order to carry Trichogramma in host eggs (grain moth) throughout the winter in Connecticut, the eggs would have to be parasitized in midfall or early in the winter, because of the possi-

bility of having them all hatch before winter if parasitized in September or early October. In nature it is possible to have continual generations, occurring throughout the winter if the emerging Trichogramma are from clusters of eggs, all of which were not parasitized the first time. If such were the case, there would be available material for the mature adults to parasitize, and thus continue to perpetuate the species until spring. Under such conditions there would probably be but one winter generation, as the rate of development is greatly reduced at low temperatures.

In conclusion, it can be said that the yellow species of Trichogramma is capable of wintering over in Connecticut in grain moth eggs, but not in the adult stage. It is self-evident from the information at hand that the parasite passes the winter in nature in the eggs of native hosts. Also, that it will complete development and emerge at temperatures over a considerable range.

POLYEMBRYONY AND PARTHENOGENESIS

Development of more than one individual from a single egg has never been observed in Trichogramma so far as we know.

Cyclic parthenogenesis occurs definitely in certain European species, but does not occur in the American species to our knowledge. The females will oviposit without fertilization, but the progeny are males.

LABORATORY STUDIES IN BREEDING AND PRODUCTION

It has already been mentioned that individuals of the several species of Trichogramma are so nearly identical in structure that they cannot be separated on the basis of known differences. A detailed study of both male and female (Figures 142, 143) under great magnification served only to emphasize this difficulty. Studies of their biology (the only alternative for distinguishing the species) were made, considering those phases which might prove valuable for identification. Studies have also been made on longevity, flight activities and effect of sulfur or other insecticides on parasitism in the laboratory. A breeding method based largely upon previous work has been developed, and is summarized on pages 708 and 741.

CROSS BREEDING EXPERIMENTS

Through the courtesy of a number of entomologists we have received Trichogramma from several sources throughout the United States and Canada. Attempts were made to cross the different stocks with one another, and the results are shown in Table

13. A few notes regarding the different stocks and their sources are needed to understand the table fully.

- A. Received from Dr. Peterson of Ohio (original source Illinois and Pennsylvania).
- B. Obtained from the Arlington Corn Borer Laboratory of the United States Bureau of Entomology through the courtesy of D. W. Jones and W. G. Bradley. Native to Massachusetts.
- E. Connecticut. Native; sent to Dr. A. W. Morrill and returned later.
- T. Connecticut. Native; recovered from field collections.
- M. West Texas; received from Dr. Morrill. Presumably native to West Texas.
- H. Georgia; received from Mr. C. H. Alden.
- K. Canada; received from Mr. Wishart. Originals of this stock were obtained from Louisiana.
- R. Arizona; received from Dr. Morrill. Presumably native to Arizona.
- X. Louisiana; received from Dr. Morrill.

The method of crossing consisted of first chilling the adults in a refrigerator, quick examination with a binocular, and then confinement of the two sexes in a gelatin capsule with fresh grain moth eggs. They were then held at 80° F. and 75 per cent humidity. See page 729.

SEX RATIO, VARIATIONS, AND CAUSES OF VARIATIONS

Trichogramma sex ratio is subject to variations under laboratory conditions. When reared continually at a constant temperature of 80° F. and 70 per cent relative humidity, females will as a rule occur in excess of the males, the ratio being about two to three females to one male for *pretiosa,* and four to five females to every male for *minutum.* Unfertilized females of both species produce only males. Temperatures below 40° F. are apparently detrimental to a normal ratio of both species when reared in grain moth eggs. At higher temperatures, 45 to 50°, there is no change in sex ratio, but there is considerable development above 46°. An increase in the number of days' refrigeration below 40° is closely correlated with a decrease in female adult parasites from that of three females to one male (when reared without periods of intervening refrigeration), to three males to one female after two months' refrigeration. This change in the sex ratio is gradual as the period of refrigeration increases. The accompanying table (14) shows the increasing percentage of male Trichogramma over females for different periods of refrigeration. From Tables 17 and 18, it is evident that there is a gradual increase in the percentage of males as the days of exposure in refrigeration increase. In all probability female Trichogramma are less resistent to low temperatures than males and thus succumb much sooner and in proportionately larger numbers than males. It will be noticed that where there was continuous rearing without intervening refrigeration, the ratio of females is slightly variable. Tables 14, 15, and 16 show the decided change

occurring in the sex ratio of the first generation removed from the hibernated generation, providing the period of refrigeration has exceeded ten days to two weeks in length. As a rule the ensuing generation reverts to a normal sex ratio if uninterrupted by hibernation at low temperatures.

Table 13. Results of Crossing Different Stocks of Trichogramma

Experiment No.	Number individuals of each strain crossed: Males	Number individuals of each strain crossed: Females	Results of cross
1	6 B	10 B	+
2	1 B	3 B	"
3	2 B	3 B	"
4	5 B	8 A	"
5	4 A	4 B	"
6	4 B	3 T	"
7	3 T	5 B	"
8	1 B	4 T	"
9	2 H	2 X	"
10	4 X	6 H	"
11	4 M	4 T	"
12	5 K	4 R	"
13	1 K	1 X	"
14	2 K	2 R	"
15	2 X	2 R	"
16	3 X	3 K	"
17	2 R	2 K	"
18	1 R	1 X	"
19	2 R	2 K	"
20	2 H	6 B	—
21	5 E	2 H	"
22	1 H	5 E	"
23	4 H	3 M	"
24	4 M	6 H	"
25	4 M	4 R	"
26	2 T	2 K	"
27	3 B	3 R	"
28	3 K	5 B	"

EFFECTS OF REFRIGERATION ON TRICHOGRAMMA

It has been found difficult to retain Trichogramma in refrigeration for any length of time without deleterious effects. It was first noted that a change in sex ratio was produced in both species, particularly when reared in grain moth eggs. Frequently this does not become apparent until the second generation following refrigeration. Such a large number of males may then develop that the ratio of increase is upset and it does not become normal again, so that quantity breeding may continue, until several generations have

TABLE 14. COMPARATIVE RATE OF DEVELOPMENT IN REFRIGERATION AND EFFECT OF REFRIGERATION ON SEX RATIO AT TWO DIFFERENT TEMPERATURES. SPECIES, *minutum*, Georgia.

Number days refrigerated	Pre-refrigeration development	Period of development	Sex ratio							
			Hibernated generation		1st generation removed		2nd generation removed		3rd generation removed	
			Male	Female	Male	Female	Male	Female	Male	Female
Temperature 38° F., humidity 60 per cent										
10	4 days	Normal	1	5.2	1.5	1	1	4.8	1	4
25	4 "	"	1	1.1	19	1	1	4	1	5
40	4 "	"	1.1	1	17	1	1	3	1	5.5
60	4 "	"	1.3	1	23	1	1	2.8	1	5
Temperature 46°–47° F., humidity 85 per cent										
10	4 days	1 hr. sooner than at 38°	1	2*	1.2	1	1	4.2	1	4.6
25	4 "	3 hrs. " " " 38°	1	2	17	1	1	3.5	1	4.8
40	4 "	4.8 hrs. " " " 38°	1	1.3	14	1	1	4	1	5.3
60	4 "	5.5 hrs. " " " 38°	1	1.3	18	1	1	4	1	5.5

*Sex ratio is not normal, due to some unknown cause in previous generation, probably light conditions. All parasitized eggs in the above investigations were from the same lot of material.

TABLE 15. TRICHOGRAMMA SEX RATIO FOR SEVERAL GENERATIONS FOLLOWING HIBERNATION.

Temperature 38° F., humidity 60 per cent

Species	Strain	Pre-hibernation development	Period of hibernation	Sex ratio of hibernated generation		Sex ration of 1st generation following hibernated generation		Sex ratio of 2d generation following hibernated generation		Sex ratio of 3rd generation following hibernated generation		Sex ratio of 4th generation following hibernated generation	
				Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Dark	Arizona	4 days	43 days	1.6	1	3	1	1	5.3				
"	Canada	4 "	43 "	1.7	1	3.3	1	1	5.6				
"	Louisiana	4 "	38 "	1.2	1	6.6	1	1	6	1	2		
Yellow	W. Texas	4 "	45 "	1	3.7	5.6	1	1	2	1	1.3	1	1.7
"	Ohio	4 "	13 "	1	2	1	3	1	1.7	1	2		
"	Mass.	4 "	13 "	1	1.6	1	1	1	1.6				
"	Conn.	4 "	13 "	1	1.9	1	2	1	2	....	....		
Dark	Georgia	4 "	43 "	1.6	1	3.1	1	1	6				
	Conn.	4 "	10 "	1	2	1.2	1	1	3.1				
	"	4 "	20 "	1	1	1.7	1	1	1.5				
	"	4 "	30 "	1.2	1	1.7	1	1	2				
	"	4 "	40 "	1.2	1	3.7	1	1	3.1				
	"	4 "	55 "	1.3	1	7	1	1.4	1				

Temperature 46° F., humidity 85 per cent

Species	Strain	Pre-hibernation development	Period of hibernation	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	Conn.	4 days	10 days	1	2	1	2	1	1.8				
	"	4 "	20 "	1	2	3	1	1	1.7				
	"	4 "	30 "	1	1.5	1.4	1	1	1.5				
	"	4 "	40 "	1	1	2.8	1	1	2.5				
	"	4 "	55 "	1	2.5	4.8	1	1	1.7				

TABLE 16. SEX RATIO OF FIRST GENERATION FROM HIBERNATED GENERATION AND OF SUCCEEDING GENERATION.
HIBERNATION TEMPERATURE 40°–46° F., HUMIDITY 60-80 PER CENT

Source of stock	Period of hibernation	Sex ratio of hibernated generation		Sex ratio of first generation from hibernated generation		Sex ratio of 2nd generation from hibernated generation		Sex ratio of 3rd generation from hibernated generation	
		Males	Females	Males	Females	Males	Females	Males	Females
Yellow species									
West Texas	20 days	1	1.5	1	1.7	1	1.2		
Massachusetts	40 "	1	1	5.6	1	1	1.2		
Connecticut	16 "	1	2	1	2	1	2.2		
"	16 "	1	2	1	2	1	2		
"	28 "	1	2.5	1.3	1	1	2.5		
Ohio	39 "	1	1.7	10	1	1	2.5		
Connecticut	12 "	1	2.8	1	1.5	1	2		
Ohio	50 "	1	1.5	3	1	1	2		
Dark species									
Arizona	20 days	1	2	6	1	1	6		
"	15 "	8*	1	1	2.5	1	2.5		
Canada	20 "	1	4	5	1	1	5		
"	15 "	6*	1	1	2	1	5.5		
Louisiana	20 "	1	2.5	3	1	1	7	1	5.3
"	20 "	1	2.5	3	1	1	7	1	3
"	33 "	1	3	11.5	1	1	6		
Georgia	36 "	1	6	2.5	1	1	4		
"	17 "	2*	1	1	5	1	3.5		

Note—*Previous generation hibernated for some time.

developed. This phenomenon together with the mortality, which may be great after prolonged exposure, often makes stocks that have been held in refrigeration practically worthless for quantity breeding. Extensive experiments with temperatures and humidities for retarding development of Trichogramma show that 46° F. and 70 per cent humidity are most suitable. The two species vary considerably in ability to withstand cold, and the host in which they are reared also makes a difference. It was found by comparison that *minutum* would survive better in grain moth eggs at 45 to 46° F. than in fruit moth eggs, but better in fruit moth eggs at 37° F.

Wing deformity is of common occurrence in Trichogramma and is proportionately dependent upon the length of refrigeration. In this connection it has also become apparent that the species considered show differences, for less deformity appears in *minutum* than in *pretiosa* after 40 days' refrigeration.

RATIO OF INCREASE; VARIATIONS AND CAUSES IN PARASITE PRODUCTION

Due to several factors the ratios of Trichogramma increase varied from time to time during the period of production. Under favorable conditions and proper handling it was possible to obtain an increase of 8 or 9 to 1. However, during most of the production period the usual increase was 4 or 5 to 1. The principal factor governing a high or low ratio of increase is the parasite sex ratio, which is normally an excess of females over males. In parasite production at this Station, deviation from the normal sex ratio was usually due to refrigeration of the parasitized laboratory host eggs.

Refrigeration of Trichogramma parasitized grain moth eggs at 38° F. resulted not only in the mortality of a large number of the parasites, but in a greater mortality of female Trichogramma than of males, thus giving an excess of males when the parasites emerged at 80° F. This increased ratio of males over females is closely correlated with the number of days of refrigeration. As the days of refrigeration increase, there is a proportionate decrease in the number of potential adult parasites; however, the proportionate decrease in females is in excess of males. Under such conditions the ratio of increase from refrigerated stock cards is very low. In a case of 50 days of refrigeration it was much less than 1 to 1. However, two successive generations in the parasite incubator (after refrigeration) brought the sex ratio up to normal. The sex ratio of the first generation obtained from the refrigerated generation is with few exceptions in excess in males after one to two weeks' refrigeration at 38° to 47° F. However, this condition is more pronounced in *minutum* than in *pretiosa,* and for both species at low rather than at high temperatures. (Tables 14, 15, 16.) Continuous incubation of Trichogramma from generation to generation without intermittent periods of refrigeration will give a ratio of females in excess of males, providing all other factors for parasitism

are normal. During the period of greatest production it is not advisable to use refrigerated material to increase a stock of Trichogramma for liberation, but to maintain a continuous turnover of one-quarter to one-fifth of the material already in incubation, thus making possible a maximum production with minimum stock.

Other factors entered into the ratio of parasite increase, all of which were due to methods of procedure in handling the parasite and not in a physiological disturbance. When parasite production was first commenced, a low percentage of parasitism was obtained because of inadequate lighting facilities and a too high temperature. Both of these factors have been discussed elsewhere and nothing need be said about them except that when the difficulties were overcome, normal parasitism resulted. Furthermore, the size of the egg cards first used, considerably lowered the ratio of increase. The cards were too large for the watch-glass parasite oviposition units, but when the size was regulated, parasitism became normal. A more detailed account of the above problems in parasite production has been given under "Methods of Handling Parasites in the Laboratory."

REPRODUCTIVE CAPACITY OF TRICHOGRAMMA

When both the light and dark species of Trichogramma are reared side by side on the same species of host eggs and under the same conditions, considerable difference is obtained in the percentage of eggs parasitized by equal stocks of the two species. The various time and temperature factors of refrigeration or retardation of any nature are conducive to such a condition, providing the stock material of each species had been previously retained under different conditions or under the same conditions for varying periods of time. However, the results after eliminating these and all other outside influences, demonstrate conclusively that the egg deposition ability of *Trichogramma minutum* and *T. pretiosa* from a purely biological standpoint is strikingly different. A thorough analysis of the data at hand shows that under laboratory and insectary conditions, the dark species of Trichogramma is 50 per cent more prolific than the yellow.

The normal sex ratio of the dark species *(minutum)* is one male to four to six females, while that of the yellow species *(pretiosa)* is one male to 1.5 to 2.5 females. All other factors being equal, this condition will naturally account for a higher rate of parasitism among strains of the dark species than among those of the yellow species. In order to arrive at the fecundity of the strains of both species, a system was worked out whereby individuals from each strain could be isolated and studied separately.

PROCEDURE FOR STUDYING INDIVIDUAL FEMALE FECUNDITY

Cards of freshly parasitized grain moth eggs for each strain of the dark and light species of Trichogramma were tapped gently over petri dishes. Only the single loose eggs that fell into the dishes were used; the remaining eggs in clusters of from two to six or eight, were returned to their respective cards. The single eggs were then transferred individually to Number 00 gelatin capsules, one to a capsule, the capsules marked, and placed in an 80° F. incubator for emergence. As the parasites emerged, the capsules were transferred to a 40° F. refrigerator for about 10 minutes, a sufficient length of time to render the adults inactive for sex determination. After an elapse of 10 minutes of refrigeration, four capsules were removed from the refrigerator at a time and examined under a binocular. In this way the sexes were accurately determined and the capsules for each strain separated into two groups, one male and one female. If it were desired to use fertilized females, one male was transferred to each capsule containing a female. This operation may be successfully done in either of two ways. The individuals may again be subjected to 40° F. after their sex has been determined (although this is not always necessary as the parasites very often remain inactive for a number of minutes after once being placed in the refrigerator) or advantage may be taken of their positive phototrophic tendency. If the former method is adopted, one sex is tapped into either half of the capsule in which it emerged and the other sex is tapped into the opposite half of the capsule in which it emerged. The two halves are then readily interchanged. When the second method of transfer for mating is used, opposite halves of the capsules in which a male and female have emerged are held in front of (up to) a light. The parasites as a rule move directly into that half of their respective capsules nearest the light. The opposite halves of the two capsules containing different sexes are then easily interchanged. Of the two methods, the former is the most satisfactory. Sometimes, in using the latter method, the parasites move faster than the operator's hands and escape during the interchange of the opposite halves of two capsules. After bringing the two sexes together, the capsules are placed in an 80° F. parasite incubator for one hour to assure mating.

Each unmated female is transferred to a watch-glass of several thousand fresh grain moth eggs by giving the capsule in which she has emerged a sudden tap with one's finger-nail. After females exposed to males have mated, they are transferred to watch-glasses of a few thousand grain moth eggs in the same manner as described for the unmated females.

All the individual females studied, either mated or unmated, were not of the same age when exposed to grain moth eggs. Their ages varied from less than one hour to 40 hours; however, the majority of the females were between four and eight hours old when given

fresh eggs. Age within the range of hours studied did not have any appreciable effect on the fecundity of the individual. Only one lot of egg material was exposed to each female parasite because of the fact that from 50 to 75 per cent of Trichogramma die within 24 hours after emerging (in watch-glasses in our parasite incubator at 80° F.). Under these conditions, one lot of egg material is ample and remains accessible to Trichogramma for a period long enough to obtain the maximum parasitism per female.

Four days after the liberation of individual females in containers with grain moth eggs, the material was examined and the parasitized eggs removed to clean watch-glasses, and placed in a lighted incubator where they remained until the parasites emerged and died. Each watch-glass was then examined separately, the number of parasitized eggs recorded, and the number of emerged males and females tabulated. (Tables 16a, 17, 18.) Whenever an unfertilized female was exposed to grain moth eggs, the resultant progeny were all males.

An examination of Table 30 will serve to demonstrate the difference in the fecundity between the dark and yellow species of Trichogramma. Strains of the yellow species used in this work were originally obtained from Connecticut, Massachusetts, Ohio, and west Texas. For the dark species, the original stocks were procured from Louisiana, Canada, Arizona, and Georgia. The maximum number of eggs parasitized by any one female of the yellow species was 29; this occurred once for the Connecticut strain and once for the Massachusetts strain. The maximum individual parasitism for the dark species was 50, which happened twice in the case of the Georgia strain. However, the average number of eggs parasitized per female for the yellow species was considerably below that of the dark species. Out of 42 trials for the dark species, 19 resulted in 30 to 50 eggs per female, while 19 out of 49 trials for the yellow species resulted in 20 to 25 eggs per female. None of the yellow strains exceeded 30 parasitized eggs per female, and only four trials were above 25 eggs. The remaining 26 trials were below 20 parasitized eggs per female. The final average was 18.6 parasitized eggs per female for the yellow species, and 29.2 parasitized eggs per female for the dark species, or approximately 50 per cent greater fecundity for the dark species.

SOME IMPORTANT BIOLOGICAL DIFFERENCES BETWEEN *Trichogramma minutum* AND *T. pretiosa*

As a rule, males emerge from host eggs in greater numbers than females during the initial appearance of the parasite. In case of darkness or in subdued light both sexes remain for a short time on the egg-clusters from which they have emerged. When the amount of light and temperature are sufficient to stimulate adults to normal activity, a large number of males remain on the eggs and

TABLE 16A. FECUNDITY OF TRICHOGRAMMA IN 80° F. INCUBATOR
YELLOW SPECIES

Strain	Female	Age females when exposed to eggs	Number eggs parasitized	Adults emerging	
				Males	Females
Connecticut	1*	4–8 hours	21	15	0
"	2*	4–8 "	9	3	0
"	3	4–8 "	23	8	9
"	4	4–8 "	24	17	3
"	5*	24 "	18	7	0
"	6*	8 "	23	11	0
"	7	8 "	19	13	1
"	8*	8 "	29	25	0
"	9*	8 "	15	11	0
"	10*	4–8 "	17	15	0
"	11*	4–8 "	13	11	0
"	12*	4–8 "	20	19	0
"	13*	4–8 "	21	17	0
"	14*	4–8 "	10	6	0
"	15*	4–8 "	18	14	0
"	16*	4–8 "	13	9	8
"	17*	4–8 "	20	17	0
"	18*	4–8 "	22	20	0
"	19	4–8 "	23	7	12
"	20*	4–8 "	19	11	0
Massachusetts	1*	4–8 "	12	10	0
"	2*	4–8 "	20	15	0
"	3*	4–8 "	18	15	0
"	4	4–8 "	20	5	11
"	5*	8 "	18	13	0
"	6	1 "	24	10	9
"	7*	8 "	16	11	0
"	8	8 "	22	9	9
"	9*	2 "	26	25	0
"	10*	4 "	14	13	0
"	11*	36 "	23	22	0
"	12*†	40 "	22	18	0
"	13*	4 "	17	17	0
"	14	36 "	18	5	9
"	15*	4 "	19	20	0
"	16*	40 "	20	18	0
"	17	40 "	17	7	8
"	18*	1 "	8	4	0
"	19*	4 "	16	13	0
"	20*	2 "	15	12	0
"	21*	40 "	8	7	0
"	22*†	40 "	29	29	0
"	23*†	36 "	23	17	0
"	24*†	36 "	26	26	0
"	25	4 "	16	9	6
"	26*	40 "	11	10	0
Ohio	1*	1 "	25	15	0
"	2*	2 "	25	15	0
West Texas	1	4–8 "	10	2	8
			Av. 18.6		

* Unfertilized females - progeny all males.
† Female confined with male 1 hour previous to egg exposure. Mating unsuccessful.

TABLE 17. FECUNDITY OF TRICHOGRAMMA IN 80° F. INCUBATOR. DARK SPECIES.

Strain	Female	Age females when exposed to eggs	Number eggs parasitized	Adults emerging	
				Males	Females
Louisiana	1	4–8 hours	26	8	12
"	2*	32 "	11	8	0
Canada	1	4–8 "	25	6	11
"	2*	4–8 "	31	24	0
"	3*	4–8 "	15	11	0
"	4*	4–8 "	35	31	0
"	5*	4–8 "	22	17	0
"	6	4–8 "	28	51	20
"	7	4–8 "	18	6	17
Arizona	1*	4–8 "	28	23	0
"	2*	4–8 "	37	34	0
"	3*	4–8 "	26	23	0
"	4	4–8 "	20	18	7
"	5	4–8 "	28	10	9
Georgia	1*	4–8 "	30	25	0
"	2*	24 "	28	25	0
"	3*	12 "	39	30	0
"	4*	24 "	48	44	0
"	5*	12 "	36	34	0
"	6*	12 "	32	28	0
"	7*	12 "	35	35	0
"	8*	12 "	23	23	0
"	9*	12 "	22	18	0
"	10*	12 "	42	40	0
"	11*	12 "	29	33	0
"	12	12 "	25	5	16
"	13	12 "	16	3	13
"	14*	12 "	11	6	0
"	15*	12 "	44	40	0
"	16*	24 "	20	17	0
"	17*	12 "	35	31	0
"	18*	12 "	38	39	0
"	19*	12 "	29	24	0
"	20*	12 "	50	49	0
"	21*	12 "	49	47	0
"	22	12 "	12	2	9
"	23	12 "	50	5	34
"	24*	12 "	39	46	0
"	25*	12 "	43	40	0
"	26*	12 "	29	26	0
"	27*	12 "	30	31	0
"	28*	12 "	25	21	0
			Av. 29.2		

* Unfertilized females - progeny all males.

mate with the females as they emerge. Males and females of both *minutum* and *pretiosa* are negatively geotropic and positively photo-

TABLE 18. FECUNDITY OF TRICHOGRAMMA IN OPEN INSECTARY. AUGUST 1932

Yellow species

Female number	Total	1st day	2nd day	3rd day	4th day	5th day	Total
1		12					12
2	17						17
3	27						27
4	19	0	dead				19
5	24	24	0	dead			24
6		33	2	dead			35
7	10						10
8	16						16
9		13	1	0	dead		14
10		20	dead				20
							Av. 19.4

Dark species

Female number	Total	1st day	2nd day	3rd day	4th day	5th day	Total
1	38						38
2	34						34
3		37	3	0	dead		40
4	10						10
5		36	dead				36
6		23	5	dead			28
7		18	0	0	dead		18
8	42						42
9	40						40
10		31	0	0	dead		31
11		42	0	dead			42
12		56	0	dead			56
13		44	4	0	0	dead	48
14		40	6	0	0	dead	46
15		31	4	0	dead		35
							Av. 36.2

tropic. However, there seems to be a more pronounced response to light by the forms belonging to the *pretiosa* species than by those belonging to the *minutum* species. *Pretiosa* does not fly as readily as *minutum,* and is more apt than *minutum* to jump when touched. In total darkness the adults of the species studied do not emerge to any extent from their host eggs for some time after they would have normally appeared had light been present. Exposing parasitized host eggs to light after having held them in darkness beyond the minimum period of development will bring practically all Trichogramma forth in from two to three hours.

Both in the laboratory and in the field, there is a marked difference in the length of the life cycle between *T. minutum* and *T. pretiosa.* The minimum initial development of *pretiosa* at 80° F. is six and one-half days, that of *minutum* at the same temperature is seven and one-fourth days. In an open outdoor insectary the length of life cycle of the two species varies considerably because of temperature changes. However, the development of both species is notably longer during the early spring, decreasing in length up to midsummer when it is shortest, and increasing again toward fall when it about equals the duration of the early spring life cycles. During 1932 there were 13 generations of *T. pretiosa* and 12 generations of *T. minutum* between April 1 and October 26. The maximum period of development in the insectary came in October and required 22 days, while the minimum periods occurred in July and early August and required nine days.

Hibernating Trichogramma in refrigeration has presented many difficulties. There is considerable variation in the ability of the two species to survive under such conditions. They are capable of withstanding low temperatures for varying lengths of time, depending upon the stage of development reached when subjected to refrigeration. When a pre-refrigeration development of four days is allowed, *T. pretiosa* (yellow species) will survive longer than *T. minutum* (dark species) at temperatures between 49° and 37° F. At 37° F. for a period of two weeks, there is less mortality in *pretiosa* than in *minutum.* However, as the period of hibernation is increased beyond two weeks, the mortality of the *pretiosa* species becomes more rapid and eventually surpasses that of *minutum.* It cannot be said at the present time that Trichogramma can be retained at temperatures below 46° F. for longer than 50 or 60 days and result in an emergence of large numbers of the parasites when again subjected to developmental temperatures. At the termination of a 50 to 60 day period of hibernation, approximately 85 per cent to 95 per cent of both species fail to emerge. At temperatures above 46° F. Trichogramma will complete development and emerge in refrigeration.

Results of refrigeration of Trichogramma parasitized grain moth eggs have been interesting. When the parasites were allowed to develop at 80° F. for two days in grain moth eggs, followed by refrigeration at 49° F., they completed development and emerged in 30 days. When they were allowed three days' pre-refrigeration development, they completed their life cycle and emerged at 49° F. in 33¼ days, and when they were refrigerated at 49° F. after attaining four days' pre-refrigeration development, they emerged in 35 days. Under the circumstances, Trichogramma cannot be held in refrigeration for over a month at the above temperature and for only a little longer at temperatures between 46° and 49° F. In concluding this part of the paper, it may be said that although it is not advisable to refrigerate at temperatures above 46° F. with the

intention of holding the parasite in a state of suspended development, it is reasonable to hold Trichogramma at 46° to 50° F. for any period less than 30 days, as the mortality is comparatively low at these temperatures (approximately 10 to 25 per cent).

Probably more important, from the standpoint of future increases in parasite material, are the changes that take place in the sex ratio of Trichogramma, directly traceable to low refrigeration temperatures. After three weeks of refrigeration at 38° to 40° F., the normal sex ratio, which is an excess of females over males, becomes reversed and the number of males increases markedly for each additional week of refrigeration. Furthermore, when Trichogramma are retained at low temperatures for more than two weeks, there is a notable change in the sex ratio of the first generation from that of the hibernated generation. This is more pronounced in the *minutum* than in the *pretiosa* species for the third and fourth week of hibernation; beyond that the results for both species are practically the same. The sex ratio becomes normal again in the following generation, providing there is no intervening refrigeration.

The normal sex ratio of *T. minutum* averages one male to four or five females; that of *pretiosa* one male to two or three females.

The ratio of increase is different for the two species of Trichogramma when reared on grain moth eggs. The maximum of eggs parasitized by a single individual of the *pretiosa* species was 29 while the maximum number parasitized by a female of the *minutum* species was 50. Likewise, the average number of eggs parasitized by females of the *pretiosa* species was considerably below that of the *minutum* species. Out of a great number of cases investigated, 86 per cent of the *pretiosa* species resulted in less than 20 parasitized eggs per female, while 50 per cent of the investigations for the *minutum* species resulted in more than 30 parasitized eggs per female. Martin (19) dissected a number of adult female Trichogramma of the *minutum* species and counted their ova. He found that the average number was 41 with a maximum of 52 and a minimum of 30. His findings closely corroborate our results.

Although fresh host eggs give the most satisfactory results when parasitized by Trichogramma, ova in various stages of development are successfully destroyed by the parasite. Frequently host eggs having practically reached complete development will produce Trichogramma and sometimes oviposition by Trichogramma is effective up to the moment of emergence of its host.

T. minutum and *T. pretiosa* are each subject to wing deformity both previous to and during periods of hibernation. This condition is at a minimum when the species are reared continuously for successive generations. However, although the ratio of increase is variable, the percentage of increase in wing deformity is continuous throughout the range of hibernation temperatures investigated for each strain of the two species of Trichogramma under discussion.

It is apparent from the foregoing studies that the various strains represent two species formerly known as the dark and yellow strains. Inasmuch as Riley has given these forms the names *minutum* and *pretiosa,* and because of the fact that there are outstanding biological differences despite the lack of structural variations, the adoption of the specific names of Riley has been proposed.[1] The more important biological differences appearing during the course of these investigations may be summarized as follows:

(1) *Pretiosa* will not cross successfully with *minutum.*

(2) *Minutum* will not survive refrigeration as well as *pretiosa.*

(3) The sex ratio of *minutum* averages 1 male to 4 or 5 females; that of *pretiosa,* 1 male to 2 or 3 females.

(4) The ratio of increase of *minutum* is greater than that of *pretiosa.*

(5) The minimum initial development of *pretiosa* at 80° F. is 6½ days; that of *minutum* at the same temperature is 7¼ days.

(6) The abdomen of the female of *pretiosa* is yellow when reared at about 80°. That of *minutum* is dark gray or blackish at all temperatures.

(7) Wing deformity is greater in *minutum* than in *pretiosa.*

ADULT MORTALITY IN TRICHOGRAMMA

Adult Trichogramma are not very long lived under laboratory conditions. As may be seen by the accompanying Table 30, an average of 88.3 per cent of the adults die within 24 hours after emerging in watch-glass oviposition units at a temperature of 80° F. and 70 per cent relative humidity. Within 36 hours practically 100 per cent mortality occurs. This condition, however, is not an indication of what takes place in nature or under hibernation conditions of low temperature and high humidity. Table 19 gives the results of the refrigeration of a number of strains of Trichogramma at two different temperatures with approximately the same percentage of moisture being present in both cases. In these hibernation investigations of adult Trichogramma most of the parasites were handled in large numbers in watch-glasses. However, some were retained during the period of refrigeration in gelatin capsules, one individual per capsule. These did not show as sudden reaction to the temperatures as did those in the watch-glasses. For the first 24 hours at 38° and 46° F. (Table 19) there was no mortality in gelatin capsules, while the mortality in watch-glasses varied from zero to 9 per cent. The total longevity at these temperatures was 17 days, with varying percentages of mortality for the different strains on the preceding days.

There is no significant difference between the results of the several strains at the same temperatures or between like strains at different temperatures, and they simply help to point out the possibility of holding adult Trichogramma for a short while at the above

[1] Conn. Agr. Expt. Sta., Bul. 338: 600-601. 1932.

mentioned temperatures without suffering complete loss of material.

It is very probable that under field conditions adult Trichogramma may live for some time, temperature and moisture conditions permitting. It is not uncommon to have adults remain alive and active in our insectary during fall and spring for from 20 to 30 days. A few adults were kept alive for approximately four months in the insectary during fall and winter months. This, however, is unusual.

TEMPERATURE, MOISTURE AND LIGHT REQUIREMENTS FOR BREEDING TRICHOGRAMMA

Temperature, moisture and light are the governing factors in the laboratory breeding of Trichogramma, a slight variation from any one of which will mean a complete upset in parasitism of the laboratory host eggs. First of all, the degree of temperature and humidity must be constant. Of the various possibilities in the range of these two conditions, 80° F. and 70 per cent humidity have been accepted for use in rearing the yellow species of Trichogramma at this Station. A variation in temperature is unsatisfactory for maximum parasite production. A rise of five degrees will not only upset the emergence of the parasite, but in addition the scheduled time of parasitism will be interfered with, fewer parasites will issue at one time, the adult parasite will die much sooner at a higher temperature, and the final result will be a low percentage of parasitism. Humidity, although very important, does not have to be absolutely constant. A five degree variation is permissible, but it is better not to have it below 60 per cent very long.

It is possible to shorten the parasite life cycle by raising the temperature, although there is no material advantage in doing so, as the adult parasite lives a much shorter time at temperatures above 80° F. than at 80 or below. The life cycle of Trichogramma at 90° F. is less than six days in length; however, the adult mortality is very high within the first 24 hours, resulting in a low percentage of parasitism. According to Flanders'[1] work on Trichogramma, there are strains that do better at temperatures above 80° F., but the species adapted for mass production and liberation in Connecticut do better at 80° F. than above this temperature. It may be of interest to state here that the parasite life cycle can be greatly lengthened by holding the temperature low; for example, at 52° F. the cycle is extended over a period of several months. The zero of development is near 46° to 47° F.

The longevity of Trichogramma after emerging from the laboratory host egg, is from one to three days, depending on the length of

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[1] Pan-Pacific Ent., VI. no. 4: 180-181. 1930.

TABLE 19. ADULT TRICHOGRAMMA MORTALITY; CUMULATIVE

Species	Number dead in the following days															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
38° F. and 60-65 per cent humidity																
Yellow, Connecticut	0	0	0	1	2.4	3.8	6.5	12	24	33	50	57	79	91	98	100
Dark, Louisiana	0	11	18	33	42	76	81	97	100	...	...	...	...	...	...	...
Dark, Georgia	3.5	12	16	20	...	27	34	45	60	72	75	...	83	...	97	100
48° F. and 60-70 per cent humidity																
Yellow, Connecticut	2.2	6.7	18	30	50	64	82	89	95	99	100	...	...	...	...	...
Dark, Georgia	1.8	9.1	11	23	...	37	40	67	78	89	95	...	97	99	100	...
Yellow, Ohio	2.9	10	29	39	47	47	52	76	87	...	97	99	100	...	...	...
80° F. and 70 per cent humidity																
Number dead in following hours																
	8	12	16	24	36	40	48	60	72							
Dark and yellow[1]	28	27	63	88	95	100	..	100	100							

[1]Average of 17 tests in watch-glasses and 8 in gelatin capsules.

time the parasite has been held in refrigeration and the degree of temperature and percentage of humidity in the incubator. It seems that refrigeration reduces the vitality to such an extent that the parasite does not live very long in the incubator after emerging. If the humidity is low and the temperature high in the incubator when the parasite emerges, it will survive less than 24 hours.

Light is essential to parasite production and without it there is very little, if any, parasitism. The underneath system of lighting is used at this Station for mass production of Trichogramma. The idea of the use of light is to draw the positively phototropic parasites to that area occupied by the laboratory host eggs. The parasite responds very readily to the proper intensity of light and parasitizes practically all of the host eggs exposed. There is, of course, a limitation to the kind of light and its intensity; likewise, the direction whence it comes and its proximity to the oviposition units must be considered.

Many types of electric light lamps were tried out: 10 W Carbon lamps; 15 W tungsten lamps, both frosted and clear white; 25 W and 50 W tungsten lamps, frosted, and 25 W and 50 W tungsten daylight lamps (blue lamps.) The most satisfactory was the 25 W daylight lamp, which is now used exclusively. All the other lamps were adequate in attracting the parasite, but none resulted in as high parasitism as the 25 W daylight bulb. Too intense illumination stimulated the parasites to too great activity, and thus they did not parasitize as many eggs as when moderately active. The 10 W lamp sufficed for one or two watch-glasses, but failed to give sufficient illumination for a large number of them. Clear and frosted white lamps produced too glaring a light which, in the higher watt lamps, tended not only to induce over-activity, but also seemed to cause the parasite to shun the light. Blue daylight lamps reproduced light conditions more nearly as they exist in nature than any other type of lamp, and of the several wattages tried, the 25 W gave the softest light and induced the highest percentage of parasitism.

The electric light lamps must not be too near the bottom of the parasite incubator, as the heat that arises from them tends to raise the temperature within the incubator three to four degrees above that at which its thermostat is regulated. A space of about 8 inches from the top of the lamps to the underside of the incubator is sufficient distance to give uniform illumination and reduce to a minimum the possibility of overheating the interior of the incubator. As a further precaution, an electric fan placed at one end of the incubator kept a constant circulation of cool air between the lamps and the bottom of the incubator.

If the light is cast from one direction only, as from one side of the incubator, the parasites will be drawn towards it, and thus into one section of the watch-glasses where they parasitize the eggs at that point, leaving the eggs in the remainder of the watch-glasses

practically untouched. For this reason the lights should be distributed beneath the incubator where their rays fall equally on all portions of the lower surface of the watch-glasses. The interior of the incubator, being painted black, gives no reflection so the parasites are drawn downward where the Angoumois grain moth eggs are placed.

METHODS OF HANDLING TRICHOGRAMMA IN THE LABORATORY

If maintained at a constant temperature of 80° F. and relative humidity of 70 per cent, the yellow species of Trichogramma (the original lot of which came from Arlington, Mass.) has in grain moth eggs a 6½ days' period of development. Likewise the yellow species of Trichogramma collected in Connecticut has a life cycle of equal length under the same temperature and humidity conditions. Stock produced from the Arlington material was liberated in Connecticut orchards during 1930, while the native Connecticut strain was being held in the laboratory for propagation.

All of the operations in the handling of Trichogramma during mass production in the laboratory should be timed exactly in order to realize the best results and maximum production of the parasite. In our experience, any deviation from the following procedure, decided upon after considerable experimentation, may result in a much smaller production of Trichogramma than it is possible to secure.

The type of watch-glass used (Figure 145) is 2½ inches in diameter outside measurement, and 2⅛ inches in diameter inside measurement. It is grooved on the lower edge while the upper edge tapers to a blunt ridge, thus allowing the upper edge of one watch-glass to fit into the lower groove of another. (Figure 145, D, E.) However, none of the glasses meshed tight enough to retain Trichogramma, so carborundum was used to grind the upper edge of one glass into the bottom groove of another, which made them absolutely tight. The inside surface of a watch-glass is slightly concave and is one-half an inch below the upper edge.

To increase the stock of Trichogramma on hand for field liberation or for any other purpose, stock cards of Trichogramma-parasitized Angoumois grain moth eggs are taken from the refrigerator, divided into quarters or sixths, placed in watch-glasses (one section per watch-glass), and incubated (See Figure 145) in darkness at 80° F. and 70 per cent humidity, until the parasites begin to emerge. The darkness is obtained by a small, black, paper-covered cage, which is kept in the large incubator where the grain moth egg deposition cans are retained. When placed in darkness, the parasites require from three to four days before issuing; this, of course, depends upon the length of time the stock cards have been held with the parasites in the parasite incubator. As a rule the period is three days. When the parasites begin to issue, the watch-glasses are removed to the shelf in the lighted parasite incubator where they

remain for from four to five hours to allow the bulk of the parasites to emerge and to mate before fresh Angoumois grain moth eggs are exposed to them. The plan of holding the stock cards in darkness is to assure a more uniform emergence of the parasite. If the cards are kept in constant light the parasites issue over a much longer period, which results in a most erratic parasitism of the grain moth egg exposed to them and ultimately an irregular appearance of the adults of the ensuing generations. In darkness the majority of the parasites will develop to the adult stage within the grain moth egg, but will not emerge until brought into light; they will then all emerge within a few hours, mate and be ready for maximum parasitism when exposed to fresh egg cards. The following plan of procedure will illustrate the method. For apparatus used see Figures 144 and 145.

PROCEDURE

(1) (2 P. M.) Fresh grain moth eggs exposed in watch-glasses to adult Trichogramma. (80° F., 70 per cent R. H.)

(2) (2 P. M. of 4th day) Parasitized eggs removed from watch-glasses and stock for increase put in 80° incubator in darkness. Surplus stock from this operation placed in refrigerator.

(3) After incubating stock for increase at 80° in darkness for three days, they are removed at 10 A. M. of 3rd day to parasite incubator and put in light.

(4) At 2 P. M. of 3rd day (7th day counting from operation No. 1) new grain moth eggs are placed with the parasites.

(5) For liberation in the orchard a card or portion thereof is sealed in a paraffin drinking cup bag clipped together as shown in Fig. 147. The wire clipped on serves as a means of fastening to the tree.

By following the above procedure, a high percentage of parasitism (80 to 95 per cent) is obtained, otherwise the percentage drops considerably. For instance, if the stock of Trichogramma is not incubated until the middle of the fourth afternoon an insufficient number will emerge on the seventh day for exposure to fresh eggs. If parasitism is then held over to the eighth day, many of the parasites that issued on the seventh day will be dead or nearly so, while those that issued between the seventh and eighth days will not be sufficiently numerous to account for a maximum percentage of parasitism.

Cards to which grain moth eggs are fastened for parasitism (and convenience in handling) are white and smooth. They are 1⅞ inches in diameter and have six to eight small triangular holes punched through them. When the time arrives for parasitism, the required number of cards are prepared. They are then covered on one side with a film of shellac, and fresh Angoumois grain moth eggs are sifted onto them. After drying for a few seconds, the cards are gently tapped on the back to shake off all eggs not adhering to

the shellac. The number of eggs per card varies between 8,000 and 10,000. It is not necessary to submerge the eggs in shellac; only a light film should be applied to the cards.

The egg cards are then allowed to dry for from one-half to three-quarters of an hour, as the alcohol fumes from the shellac are deadly to the parasite. The egg cards are now placed in the watch-glasses of emerged adult parasites. This is done by gently raising the top of the watch-glass with the palm of the left hand, while holding an egg card (eggs downward) between the thumb and first finger of the same hand. With a pair of tweezers in the right hand, the

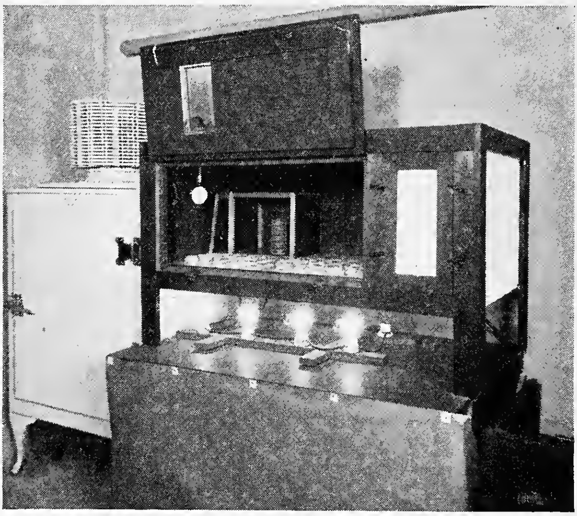

FIGURE 144. Trichogramma incubator with watch-glass breeding containers and dark box for preventing emergence before the desired time.

quarter section of stock card is raised out of the watch-glass. The egg card is then lowered into the watch-glass, the section of stock card placed on top of it (eggs upward), and the cover of the watch-glass replaced. The triangular holes in the egg cards allow sufficient light to pass up through the watch-glass to attract the parasites down to the area of fresh eggs. Egg cards must come in contact with the bottom of the watch-glasses in order to have the parasite work on the entire area of the card. Much difficulty was experienced in obtaining a uniform parasitism on the cards. The edges of the cards would be completely parasitized while the center remained untouched. It was found that the cards were too large to touch the bottom of the watch-glass, being held up by its side.

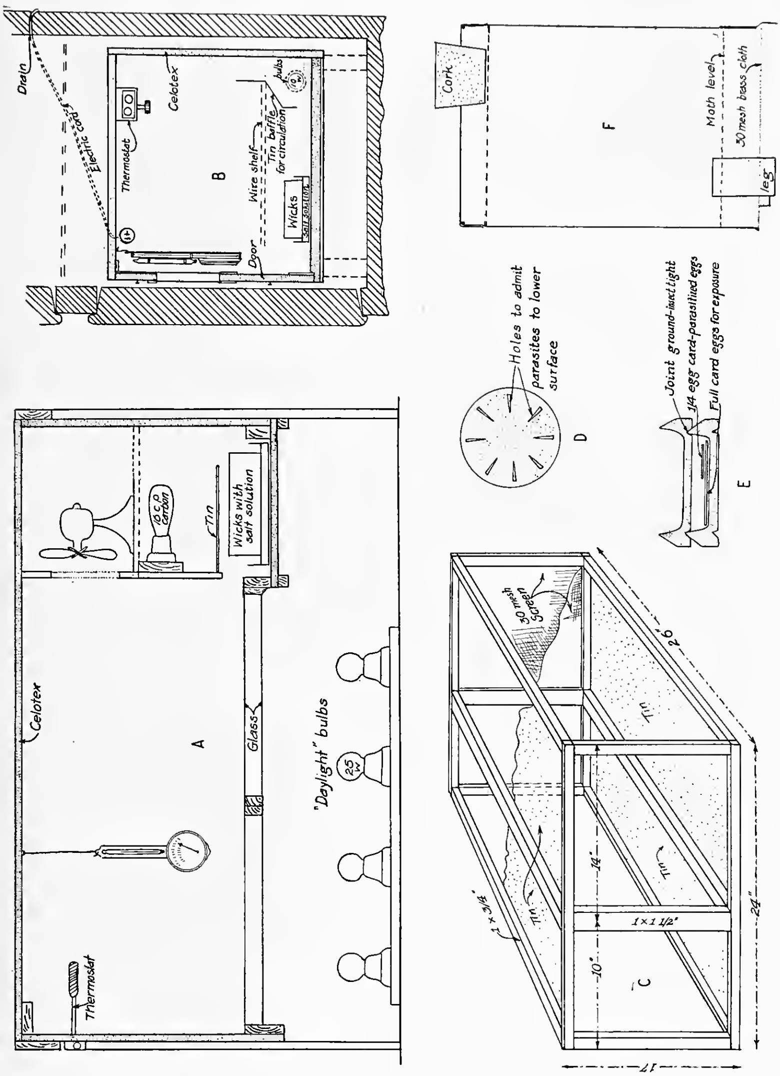

FIGURE 145. A, cross section of Trichogramma incubator shown in Figure 144, giving details of construction. B, cross section of incubator used in one of our electric refrigerators for keeping constant temperature below room temperature. C, details of grain moth breeding cage. The right hand compartment is provided with shallow trays as in Figure 139, and a funnel is fitted to the front to facilitate moth extraction. D, disc for grain moth eggs which are exposed to parasites in the watch-glass containers shown in E. F, details of the grain moth oviposition can. showing also the amount of moths by volume supplied to each can.

After the cards were trimmed down so that they rested on the bottom of the watch-glass, parasitism was uniform over the whole egg surface of the card. It is also advisable to bend an egg card so that it will fit into a slightly concave watch-glass, so that it will coincide with the concave bottom of the watch-glass. The nearer the eggs on a card can be brought to the surface over which the parasites are moving, the more uniform will be the parasitism.

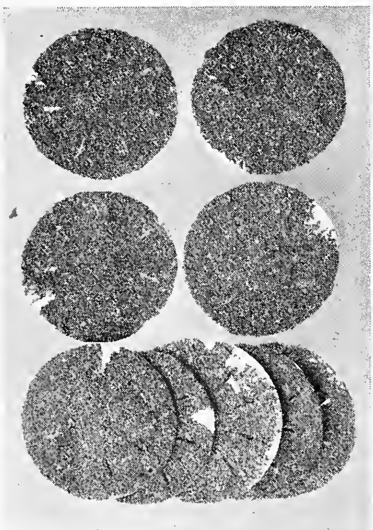

FIGURE 146. Discs with parasitized grain moth eggs. These are usually clipped into several sections and a portion placed in each paper bag as shown in Figure 147. One disc is estimated to have 8 to 10,000 parasitized eggs.

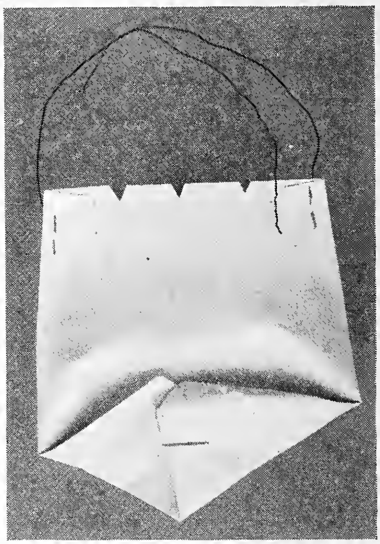

FIGURE 147. Paraffined drinking cup bag for release of Trichogramma in orchards. Small holes at the top are for escape of the parasites, and wires provide an easy means of fastening the bags to the trees.

ANNUAL PRODUCTION

Our annual production of Trichogramma is given in Table 20. The first year egg cards were sent out without any protecting device and were hung in the trees by the growers. Directions for handling them accompanied each shipment. Cards returned during the summer, however, showed so much destruction by predatory insects, evidently before the parasites emerged, that paraffin bags were prepared and portions of the cards placed in each. A wire was clipped to each bag so that they could be easily hung in trees (Fig-

ure 147.) Examination of these shipments during 1932 were much more encouraging, so they were continued in 1932. During 1931 the bags were stapled together with a stapling machine, but in 1932 they were paraffined more heavily and only enough staples employed to hold the wires in place and to close the container at the bottom.

TABLE 20. TRICHOGRAMMA PRODUCTION 1930 TO 1932

Season	Estimated Trichogramma shipped	Number shipments
1930	6,540,000	159
1931	11,337,000	282
1932	18,000,000	172

The figures for 1931 and 1932 include a considerable number of Trichogramma used for experiments, but in both years more parasites were shipped to growers than in 1931. In 1931 a large number of recoveries were made from eight orchards where Trichogramma had been liberated and in orchards where no liberations were made. These results are shown in Table 21, giving a parasitism of 23 per cent for those orchards where Trichogramma were not liberated and 45 per cent in those where they had been liberated. Table 20 shows our annual production between 1930 and 1933.

TABLE 21. PERCENTAGE OF TRICHOGRAMMA PARASITISM IN SEVERAL CONNECTICUT ORCHARDS, 1931

Orchard	Per cent parasitized eggs	Liberations
W. F. Platt, Milford	37	1930, 1931
Root, Farmington	68	1930, 1931
Kneuer, Guilford	25	none
Conn. State College, Storrs	15	1930
Avery, Yantic	45	1930, 1931
Pero, South Manchester	55	1930, 1931
Station Farm, Mount Carmel	44	none
Homewood Farms, Greenwich	0	none
Average parasitism in orchards where Trichogramma was *not* liberated		23 per cent
Average parasitism in orchards where Trichogramma *was* liberated		45 per cent

FIELD STUDIES

Experiments and observations have been made during the last three years to determine three points: First, the effect of insecticides or fungicides upon the life activities of the Trichogramma; second, how far it will travel in an orchard; and third, what can be expected in the way of parasitism from mass liberations, either made at one time or throughout the season. The flight activities observed in 1931 have already been reported.[1] These tests were repeated in 1932 with similar results, but without the high degree of parasi-

[1] Jour. Econ. Ent., 25: 370-374. 1932.

tism that occurred in 1931. Our results regarding the third point are incomplete, although the 1932 results only serve to emphasize those obtained in 1931, namely that parasitism cannot be pushed upwards beyond a certain point with any amount of liberated parasites or method of liberation.

FLIGHT OF TRICHOGRAMMA

In the past few years mass production and liberation of Trichogramma has raised the question as to whether Trichogramma is able to disperse to any great extent from points of liberation. Assuming it is carried from one location to another by wind and air currents, it is desirable to know whether it flies for any great distance, or spreads entirely on wind and air currents.

It has been noticed in peach orchards that the parasitism of fruit moth eggs early in the spring is often highest in the rows of trees nearest the woods or brushland. Due to orchard practice, few Trichogramma successfully survive the winter in peach orchards. Thus the parasitism of fruit moth eggs for the succeeding year depends upon the spread of the parasite from areas outside the orchards in which they passed the winter in native host eggs.

Taking these facts into consideration, it was thought advisable to make observations on the spread of Trichogramma from a standpoint of flight, both under laboratory and field conditions.

Several hundred Trichogramma were liberated in a dark room where there were no air currents. The point at which the parasites were liberated was five feet distant from a light. Three minutes after liberation, Trichogramma were seen crawling around on a white card back of the light. A second trial was made in a dark cellar where the temperature was 57° F. At a point 24 feet distant from a light about 400 Trichogramma were liberated. One female flew the distance of 24 feet in two minutes; five minutes later a male parasite was seen on the cardboard back of the light.

Following the laboratory experiments, field work was conducted to obtain information on the spread of the parasite under natural conditions. The orchard in which the work was conducted contained 300 twelve-year old trees planted 18 feet apart in rows running north and south. A chance collection of fruit moth eggs was undertaken in August, but none were found due to the scarcity of fruit moths. An experimental block of 121 trees was used in the center of the orchard and from this a cross of 21 trees through the center was selected for specific observations. From this cross, data were secured and used in figuring the results of the experiment throughout the entire block.

Fruit moth eggs deposited on peach leaves were obtained in the laboratory and distributed in the 21 trees. Three days later the eggs were collected and found to be unparasitized. A check on this result showed the condition to be the same in another portion

of the orchard and was considered to be sufficient evidence of the absence of Trichogramma. Flour sacks containing 40 fruit moths each were planted in each of the 21 trees of the cross. Three days later they were transferred to 21 different branches of the same trees and 10 bags containing sections of Trichogramma-parasitized grain moth egg cards, were hung in the center tree of the cross. The eggs, from which parasites were emerging, totalled approximately 20,000. Three days after liberating the Trichogramma the branches were cut from the trees and an examination of the leaves made for parasitized fruit moth eggs. Tables 22 to 23 give the count before and after liberation. The radius of spread computed from the average percentage of parasitism was greatest to the west. The prevailing winds during the first part of the experiment was from the north for the first day, south for the second, and southwest for the third day. The average wind velocity for the period was 7.7 miles per hour and during the three days the wind shifted to the east for only five hours—three hours to the northeast on the first day and two hours to the southeast on the second.

The prevailing wind during the second part of the experiment was northwest during the first day, west during the second day, and north during the third day. The average wind velocity for the period was 5.6 miles per hour. The only time the wind shifted to the east was for four successive hours during the late morning and early afternoon hours of the third day. In spite of the fact that the wind, during the time the eggs for the first trial were exposed, was blowing from the southwest and from the northwest during the exposure of the fruit moth eggs for the second trial, the average percentage of parasitized eggs was higher in the western half of the block than in the eastern half.

Taking both of these trials and averaging their results, the highest average percentage of parasitized eggs is found to the west for a single direction, 61 per cent. The average wind velocity for both periods of 6.7 miles per hour from a general westerly direction, did not account for a higher parasitism in the eastern half of the block; furthermore, it was not strong enough to interfere with the spread of the parasite to the west as there was a higher average percentage of parasitism in the western half of the block. The distance between each two trees being 18 feet, the total area the experiment covered was 32,400 feet, or about three-fourths of an acre. How far outside of the area the parasite dispersed is not known, but, judging from the percentage of parasitized eggs on the outer trees, it is safe to say the parasite spread considerably beyond the outside boundary of the experimental block of 121 trees It seems reasonable to believe the spread of Trichogramma throughout the block and beyond was in part due to the flight of the parasite. Air currents and wind exerted some influence, as the results indicate, but it seems evident the Trichogramma is capable of flying a considerable distance.

TABLE 22. FRUIT MOTH EGG PARASITISM BEFORE TRICHOGRAMMA WERE LIBERATED

Tree	Number eggs	Per cent parasitism
1	10	0
2	12	0
3	18	0
4	8	0
5	32	0
6	11	0
7	5	0
8	7	0
9	18	0
10	15	0
11	78	0
12	14	0
13	27	0
14	26	0
15	115	0
16	92	0
17	24	0
18	85	0
19	33	0
20	18	0
21	8	0
	Total 656	Av. 0

EFFECT OF SULFUR ON EGG PARASITISM

We have observed in peach orchards in which the spray schedule used throughout the season included sulfur, that there was a low percentage of parasitized fruit moth eggs. Having previous information regarding the adverse effect of sulfur on Trichogramma exposed to eggs obtained from Angoumois grain moths reared in sulfured grain, we were convinced that the low percentage of fruit moth egg parasitism was due in part to the use of sulfur as a combined insecticide and fungicide.

In 1930, several experiments were conducted to verify the above supposition. The results of these experiments were not conclusive.

To clear up these points, we have conducted laboratory tests with the use of sulfured fruit moth and Angoumois grain moth eggs for Trichogramma parasitism.

One of two small potted peach trees containing fruit moth eggs was dusted with fine dusting sulfur, while the second was held as a check. A glass jar was inverted over each peach seedling and a quartered section of an egg card from which the yellow species of Trichogramma had begun to emerge was exposed under each jar with the seedling. The seedlings were kept in the greenhouse where the temperature fluctuated between 58 and 81° F. Several days

TABLE 23. FRUIT MOTH EGG PARASITISM AFTER TRICHOGRAMMA WERE LIBERATED

Tree	Number eggs	Per cent parasitism	Tree	Number eggs	Per cent parasitism
1	3	0	1	5	0
2	0	0	2	0	0
3	94	81	3	0	0
4	2	50	4	6	50
5	6	66	5	8	50
6	3	66	6	9	55
7	72	87	7	8	62
8	0	0	8	4	50
9	0	0	9	0	0
10	20	45	10	69	30
11	28	14	11	38	34
12	34	91	12	46	52
13	5	0	13	49	91
14	10	70	14	14	78
15	3	66	15	13	46
16	0	0	16	0	0
17	10	50	17	6	50
18	9	22	18	22	90
19	20	25	19	19	63
20	0	0	20	0	0
21	2	50	21	40	37
	Total 321	Av. 48		Total 356	Av. 52

later, the eggs were examined and found to be 100 per cent parasitized on both the sulfured and unsulfured seedlings.

Four more seedlings were prepared, two sulfured and two unsulfured. One pair was placed in an 82° F. incubator and the other pair in the greenhouse where the average temperature was 72° F. with a maximum of 81° and a minimum of 60°. At the same time, four cards of Angoumois grain moth eggs were prepared. Two were dusted with sulfur and two were held without the use of sulfur. One pair was placed in the 82° F. incubator, while the second pair was placed in the greenhouse. In the greenhouse, where the average temperature was below 75° F. and the humidity 40 per cent, the sulfured fruit moth eggs were 57 per cent parasitized, while the

TABLE 24. PARASITISM BEFORE AND AFTER TRICHOGRAMMA LIBERATION

	Egg count	Per cent parasitism
Before	656	0
Three days after	321	48
Six days after	356	52

check showed 100 per cent parasitism. On the other hand, the sulfured grain moth eggs were but 8 per cent parasitized as compared with 100 per cent parasitism for the unsulfured grain moth eggs. The results for grain moth and fruit moth eggs combined give an average parasitism of 32.5 per cent, while the checks were 100 per cent parasitized. The sulfured fruit moth eggs in the 82° F. incubator, where the humidity was 75 per cent, were 31 per cent parasitized and the unsulfured eggs were 100 per cent parasitized. The grain moth eggs were 6 per cent parasitized for the sulfured lot and 100 per cent for the unsulfured lot. An average of the combined results for grain moth and fruit moth eggs was 22 per cent parasitism for sulfured eggs and 100 per cent for unsulfured.

It is probable that sulfur will not only repel Trichogramma when the temperature averages above 70° F., but it likewise has a killing effect in proportion to the increase in temperature. This is especially true where the parasites are confined in small receptacles as in the case of watch-glasses. Here, as the sulfur volatilized, it had no means of dissipation, and thus increased in strength and killed the parasites. In the jars inverted over the peach seedlings, the kill from volatilized sulfur was not so great because the sulfur vapor had a greater area in which to dissipate and thus had a less toxic effect on Trichogramma. However, at temperatures below 80° F. there is less killing effect than at temperatures above 80°. There is no doubt that when the average temperature in peach orchards where sulfur is used during peach moth egg deposition periods is 80° F. or above, there will be less peach moth egg parasitism than in orchards where sulfur is not used.

The higher the temperature within the limits mentioned, the more readily sulfur will volatilize. There is sufficient volatilization of sulfur at 80° F. and higher to repel or kill Trichogramma almost completely and thus result in practically no parasitism of grain moth and fruit moth eggs. Below 80° F., there is some volatilization, diminishing with a decrease in temperature. A reduction in volatilization is accompanied by less and less killing effect until a point is reached where there is little or no volatilization and a normal parasitism results.

In 1932, the experiments were repeated with fruit moth eggs, using covered cages through which a constant interchange of air could be maintained. In conjunction with sulfur, talc, oil dust, lime and a combination dust were used. An examination of Table 25 will serve to demonstrate the outcome of these investigations. The average parasitism of fruit moth eggs for each treatment was 40 to 50 per cent, except in the case of talc, which was 9.5 per cent. Talc applications were the most harmful of all the treatments for Trichogramma. Tables 26, 27, and 28 show effects of sulfur on the percentage of eggs parasitized under orchard conditions.

TABLE 25. EFFECT OF ORCHARD DUSTS ON TRICHOGRAMMA PARASITISM OF ORIENTAL FRUIT MOTH EGGS. GREENHOUSE EXPERIMENTS[1]

Treatment	Number tests	Average temperature	Average humidity per cent	Average parasitism per cent
Sulfur 90 per cent	43	79° F.	56	50.2
Combination 75% sulfur, 20% lime, 5% lead arsenate	29	80° F.	70	48.7
High calcium lime	15	75° F.	70	43.9
Oil dust 90% lime, oil, lead arsenate	21	79° F.	78	48.0
Fibrous talc	31	78° F.	69	9.5

[1] Control experiments showed 100 per cent parasitism in nearly every case.

TABLE 26. TRICHOGRAMMA PARASITISM IN PEACH ORCHARD WHERE SULFUR DUST[1] WAS USED SIMULTANEOUSLY WITH THE LIBERATION OF THE PARASITES

Check or dusted	Tree number	Number eggs taken	Number parasitized	Per cent parasitism
Check	7	64	16	25
"	8	0	0	0
"	9	25	5	20
"	10	8	4	50
Dusted	1	17	1	5.8
"	2	22	2	9.9
"	3	26	1	3.8
"	4	17	0	0
"	5	3	0	0
"	6	6	0	0

[1] Sulfur dust, 3 pounds per tree.

Total percentage parasitism on check trees	25.7
Average percentage parasitism on check trees	31.6
Total percentage parasitism on dusted trees	4.3
Average percentage parasitism on dusted trees	3.2

TABLE 27. TRICHOGRAMMA PARASITISM IN PEACH ORCHARD WHERE A SULFUR SPRAY[1] WAS USED SIMULTANEOUSLY WITH THE LIBERATION OF THE PARASITE

Sprayed or check	Tree number	Number eggs	Number parasitism	Per cent parasitism
Check	10	38	9	23.6
"	7	23	2	8.6
"	9	15	0	0
"	8	51	30	58.8
Sprayed	1	2	0	0
"	4	1	0	0
"	3	5	0	0
"	6	37	0	0
"	5	35	3	8.5
"	2	23	0	0

[1]Sprayed { 10 lbs. of flotation sulfur / 4 lbs. of lime } —100 gals. water.

Total percentage parasitism on check trees	32
Average percentage parasitism on check trees	22.7
Total percentage parasitism on sprayed trees	2.9
Average percentage parasitism on sprayed trees	1.4

EFFECT OF TRICHOGRAMMA PARASITISM AND LIBERATIONS ON FRUIT MOTH INFESTATIONS

It was observed in 1931 that low infestations of the fruit moth were nearly always accompanied by high parasitism of Trichogramma and Macrocentrus. Which of these parasites was responsible, could not be determined easily because they were nearly, if not always, associated. So far as could be seen in 1932 and 1931 by field collections in the orchard of the Connecticut State College, only Trichogramma was present, but for some reason was low in numbers. A study of this orchard was then begun and in 1932 1,250,000 Trichogramma were liberated at intervals of two weeks throughout the season. Heavy applications of sulfur were made in this orchard both in 1931 and 1932, those in 1932 continuing until August. As a general result, parasitism was again low in 1932 during the middle of the summer when the second brood was at its peak, although it averaged higher than in 1931. The general infestation was much lower than in 1931, but was still too high to be called commercial control. Some of the results are shown in Tables 29 and 30, although no conclusions will be drawn from them at this time.

TABLE 28. NATURAL PARASITISM OF PEACH MOTH EGGS IN SPRAYED BLOCKS

Tree	Number eggs parasitized	Number eggs not parasitized	Per cent parasitism	Notes
1	18	21	46.1	Check trees. Average per cent parasitism, 14.8.
2	1	12	7.6	
3	3	13	18.7	
4	0	2	0.0	
5	3	12	2.0	
6	8	70	10.2	Sprayed flotation sulfur. Average per cent parasitism 5.6.
7	0	14	0.0	
8	1	24	4.0	
9	0	14	0.0	
10	2	12	14.2	
11	1	12	7.6	Check trees. Average per cent parasitism, 6.5.
12	0	0	0.0	
13	0	9	0.0	
14	0	0	0.0	
15	1	3	25.0	
16	0	16	0.0	Sprayed 10 lbs. flotation sulfur, 4 lbs. lime to 100 gals. Average per cent parasitism, 18.8
17	1	6	14.2	
18	1	18	5.2	
19	4	12	25.0	
20	1	1	50.0	
Average percentage parasitism of combined checks. . . .			10.1	
Average percentage parasitism of combined sprayed and dusted.			12.2	

TABLE 29. EFFECT OF TRICHOGRAMMA LIBERATION ON FRUIT MOTH EGG PARASITISM

1931

Date	Number parasites liberated	Per cent of fruit moth egg parasitism	
		Previous to liberation	Following liberation
August 6	40,000	5 per cent	58 per cent

1932

Date	Number parasites liberated	Previous to liberation	Following liberation
June 6	54,000	40 per cent	40 per cent
June 21	54,000		84 " "
July 5	54,000		10 " "
July 19	54,000		47 " "
August 2	356,000		46 " "
August 16	640,000		50 " "
			Av. 53.4 " "

TABLE 30. EFFECT OF TRICHOGRAMMA PARASITISM ON FRUIT INFESTATION

1932

Orchard and location	Date picked	Per cent infestation	Average per cent Trichogramma parasitism; seasonal
E. A. Smith, Hebron	Sept. 8	9.4	58
Conn. State College, Storrs	Sept. 8 Sept. 19	38.2 64.3	53
Savage, Storrs	Sept. 8	38.8	30
1931 and 1932			
Conn. State College, Storrs	1931	80	15
Conn. State College, Storrs	1932	50	53

GENERAL SUMMARY

THE GRAIN MOTH

1. The grain moth was used as host throughout these experiments in breeding Trichogramma.

2. Red winter wheat was found to be satisfactory for rearing the grain moth and special units for handling moths were constructed. Breeding was carried on at about 80° F. and 70 per cent relative humidity.

3. Infertile eggs may be caused by too frequent extraction of moths.

4. Eggs are separated with sieves, mites eliminated by frequent extraction from units, and various manipulations used to free the eggs from foreign materials.

5. Shellac was found to be the most satisfactory material for fastening the eggs to cards.

6. Refrigeration in electric refrigerators is deleterious to grain moth eggs and renders them unfit for reinoculation of cages. They may be used for parasitism.

7. Too high temperatures, above 85° F., are not desirable in cages producing moths.

8. Depletion of grain was found to be 70 per cent in 1930, with 4½ pounds per tray, and 92 per cent in 1931, with 3½ pounds per tray.

9. The chief enemies of the moths are ants and mites of the family Parasitoidea. Grain beetles compete for food if they become abundant. Ants have been controlled with repellents, and mites and beetles by preheating the grain and by frequent extractions.

10. Experiments were conducted in heating the rooms to destroy mites without destroying grain moths.

11. The general procedure used for moth production is given on page 708.

THE EGG PARASITES

12. Habits and life history of Trichogramma have been studied. Many hosts are parasitized. Adults live from 5 to 119 days in the insectary.

13. The maximum number of generations was 15. They were successfully wintered in grain moth eggs from November 6 to April 3.

14. Polyembryony has not been observed. Unfertilized females deposit eggs that produce only males.

15. Cross breeding experiments indicate that there are at least two distinct species of Trichogramma. *T. pretiosa* is most commonly encountered in Connecticut orchards.

16. Refrigeration has been observed to affect the sex ratio particularly of the generation following refrigeration.

17. Experiments indicate that 46 to 47° F. is best for keeping parasitized material in grain moth eggs.

18. Trichogramma apparently lived better in grain moth than in fruit moth eggs at 45 to 46° F., but survived better in fruit moth eggs at 37° F.

19. Wing deformity is dependent on the length of refrigeration.

20. Rate of increase is dependent on sex ratio, and methods of handling, such as position of egg cards, light, etc.

21. One female is capable of laying 18 to 29 eggs under favorable conditions.

22. Temperature, moisture and light requirements are described and methods of handling the parasites are given, pages 739, 740.

23. Annual production in our laboratory varied from 6,000,000 in 1930 to 18,000,000 Trichogramma in 1932. The general procedure is given on page 741.

24. The average percentage parasitism in 1931 in three orchards where no Trichogramma were liberated was 23; in those orchards where they were liberated it was 45 per cent.

25. Trichogramma liberated in quantities at a single point dispersed over an area of 32,400 square feet. They were proved capable of direct flight by laboratory experiments.

26. Sulfur affects the amount of parasitism if applied at the time of liberation. In laboratory tests it reduced the parasitism about 50 per cent. Talc dust was more injurious than sulfur, sulfur-lime-lead arsenate dust, lime dust, or lime-oil-lead arsenate dust.

27. Field experiments indicated that liberations of Trichogramma raised parasitism and kept it about 50 per cent, even though frequent sulfur applications were made. It is believed that an interval of a week or 10 days between spray application and parasite liberation will reduce the injurious effect of sulfur to a minimum.

28. Observations and field counts indicated that high Trichogramma parasitism was correlated with reduced infestation, but the reduction was not enough in some cases to be called commercial control.

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